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J. J. C. BUCKLEY, D.Sc.
William Julien Courtauld Professor of Helminthology
in the University of London

London School of Hygiene
and
Tropical Medicine
Keppel Street
London
W.C.1

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On the Adult Morphology of *Wuchereria* sp. *(malayi ?)* from a Monkey *(Macaca irus)* and from Cats in Malaya, and on *Wuchereria pahangi* n.sp. from a Dog and a Cat

By J. J. C. Buckley
London School of Hygiene and Tropical Medicine
and
J. F. B. Edeson
Institute for Medical Research, Kuantan, Malaya

In a recent communication, Edeson, Wharton and Buckley (1955) reported on the occurrence of microfilariae resembling those of *W. malayi*, in the blood of dogs and cats and in three different kinds of Primates namely the Kra monkey (*Macaca irus*), the Lotong (*Presbytis melalophos*) and the Slow Loris (*Nycticebus coucang*). The infected animals came from areas of endemic human filariasis due to *W. malayi* in East Pahang. They also reported that adult worms of the genus *Wuchereria* had been recovered from the lymphatic systems of the Kra monkey, the dog and cat. The present paper is mainly concerned with descriptions of the morphology of these adult worms.

Material and Methods

In searching for adult worms in animals showing *malayi*-like microfilariae in their blood, the method adopted was based on the assumption that, as in cases of *Wuchereria* infections in man, the most likely habitat for the worms was the lymphatic glands or vessels. After skinning and opening up the abdominal cavity all the lymphatic glands which could be found together with the associated vessels and tissues were removed and placed in labelled Petri dishes containing normal saline. Small glands were examined direct after being teased out with a pair of mounted needles under a binocular microscope, and if no worms were observed the material was squashed in a "compressorium" consisting of two pieces of plate glass measuring 3 in. by 4 in. and examined again. Larger glands were cut up into suitably small pieces and examined in the same way. The use of the compressorium proved to be a most important factor in revealing the presence of worms which owing

to their small size and the opacity of the surrounding tissues would otherwise have been overlooked. On a very few occasions adult worms or fragments were found in the Petri dish having migrated from the tissues before these were examined in the compressorium.

Adult worms or fragments were collected post-mortem from 7 animals, namely Kra monkey (1), dog (1), cats (4) and Slow Loris (1). Their recovery represented much time-consuming, tedious and often unrewarding work, for it involved the examination of much glandular material that proved unproductive of worms and of animals in which no worms could be found although microfilariae were present in the blood. Animals in which there was an abundance of fatty tissue surrounding the glands were particularly difficult to work with for not only were the worms obscured by it but the glands themselves were sometimes difficult to isolate.

Adult worms were transferred to a watch glass with a small quantity of normal saline and were fixed by adding hot 70% alcohol. A small quantity, about 10%, of glycerine was then added and the alcohol was allowed to evaporate until the worms were in pure glycerine. Each specimen was mounted separately in glycerine as a permanent hanging-drop preparation on a cavity slide, which made for ease of examination and avoided the risk of damage by excessive handling and cover-glass pressure.

Wuchereria sp. (*malayi?*) from *Macaca irus*

A Kra monkey (No. IV) which had a natural infection in its blood with *malayi*-like microfilaria and had rendered most useful service during months of captivity at the Kuantan laboratory in mosquito-feeding experiments, died on 30th August, 1955. The microfilarial density was low (maximum 75 in 60 c.mm. of blood) but showed a markedly nocturnal periodicity. A post-mortem examination and search in this animal, which kept two workers fully occupied for two days, resulted in the recovery of 9 adult *Wuchereria*, 5 males and 4 females, all in perfect condition. One female worm, rather immature, and one male were obtained from an abdominal gland and the remaining seven were found lying in a lymphatic vessel alongside one of the spermatic cords. The recovery of this valuable material was particularly fortunate in view of the difficulty of obtaining naturally infected monkeys. Of 55 (*Macaca irus*) previously examined for microfilariae only 3 had been found positive. Post-mortem examination of two of these animals yielded no adult worms.

Morphology

The female

The principal dimensions of the four female worms are indicated in Table I. The body *cuticle* is unstriated and smooth throughout except in the tail region where it bears very minute cuticular bosses or tubercles as described in the female of *W. bancrofti* (Buckley, 1952). They can be seen only under a high magnification (Fig. 5) and anterior to the anus they become sparse and inconspicuous and disappear altogether within a few millimetres from the tip of the tail.

The *anterior extremity* is globular (Figs. 1 and 2) and bears 10 papillae in two rows, 6 circumoral comprising one pair situated laterally and two pairs in the sub-dorsal and sub-ventral position. Behind this row is a row of 4 sub-lateral papillae. This arrangement is identical with that described for *W. bancrofti* (Buckley, 1952a) but the papillae in *W. bancrofti* are relatively smaller and less prominent than in the present specimens.

The *oesophagus* is cuticularized at the mouth opening (Fig. 2). It increases very gradually in diameter posteriorly to its junction with the intestine. The junction of the muscular with the glandular portion is not very clearly seen but the muscular part is about $\frac{1}{3}$ the total length. The *excretory pore* and *nerve ring* are very difficult to discern.

The *vulva* is a transverse slit and from it the narrow, cuticle-lined lumen of the pear-shaped *ovejector* runs back and then forward again before entering the vagina. At the first bend the lumen is thrown into complicated folds or convolutions. The *vagina* is a long double-walled tube with a rather narrow lumen in which microfilariae can be seen. It may be coiled on itself before entering the *uterus* which in specimen No. 1 (Fig. 1) is a wide tube tightly packed with microfilariae, which does not bifurcate into the two uteri for some distance posterior to its junction with the vagina.

The male

The principal dimensions of the 5 male specimens are indicated in Table I, and their tail regions are illustrated in Figs. 6—12.

The anterior extremity and cuticle are the same as in the female. In the tail regions the cuticle is smooth but on the ventral aspect

there may be pseudo-striations due to the coiling of this part of the body. There are 2—3 coils which in all the specimens form a left-handed spiral (i.e. in the opposite direction to a corkscrew). This explains why Figures 6—10 illustrate only the left side of the tail region. It is impossible, due to the coiling, to view this region from the right hand side without cutting off the last coil and mounting it in the reverse position. One specimen only was sacrificed for this purpose and also to ascertain the number and arrangement of the adanal papillae (Figs. 11 and 12). The *caudal papillae* may be grouped as follows : adanal, intermediate and terminal. Seen from the left side, the left lateral adanal papillae numbered 3, 4, 3, 3, 4 respectively in the five specimens. (Figs. 6—10). They are large but not all equal in size (e.g. Fig. 7). At a deeper focus, just behind the anus, one or more post-adanal papillae could usually be seen and the sub-ventral view of one specimen (Fig. 11) revealed that these comprise a pair side-by-side just behind the anus. This view also revealed 4 lateral adanals on the right side. It may be assumed that the typical arrangement may be designated as follows : 3 to 4 lateral adanals on each side and one pair of post adanals. Just in front of the anus is a prominence which possibly represents an unpaired pre-anal papilla. The terminal group of papillae (at the tip of the tail) numbered 3, 3, 2, 2, 2, respectively, on the left side in the 5 specimens. These are smaller in size than the adanals and sometimes difficult to count. It may be assumed that the terminal papillae are characteristically 4 to 6 in number. The intermediate group of papillae are also variable in position and in number, from 0 (Fig. 7) to 2 (Fig. 6) on the left side. In size they vary between that of the adanals and the terminals, e.g. the anterior papilla in Fig. 6 is distinctly larger than the posterior one.

The *spicules* are as follows : The longer left spicule is in 3 parts, first a tubular proximal part, open and slightly expanded at its proximal end. It is less than half the total length and has a pitted appearance ("granular stippling") which distinguishes it clearly from the median and distal parts. The second or median part is short and non-tubular. It is difficult to describe its appearance but it might be likened to one half of a leaf bisected longitudinally whose median stem is a prolongation of the tubular proximal part of the

Wuchereria sp. (*malayi*?) from *Macaca irus*.

Fig. 1.—Anterior portion of female. Fig. 2.—Sub-ventral view of head. Fig. 3.—End-on view of head (schematic). Fig. 4.—Vulva, ovejector and part of vagina. Fig. 5.—Tail of female, showing cuticular bosses.

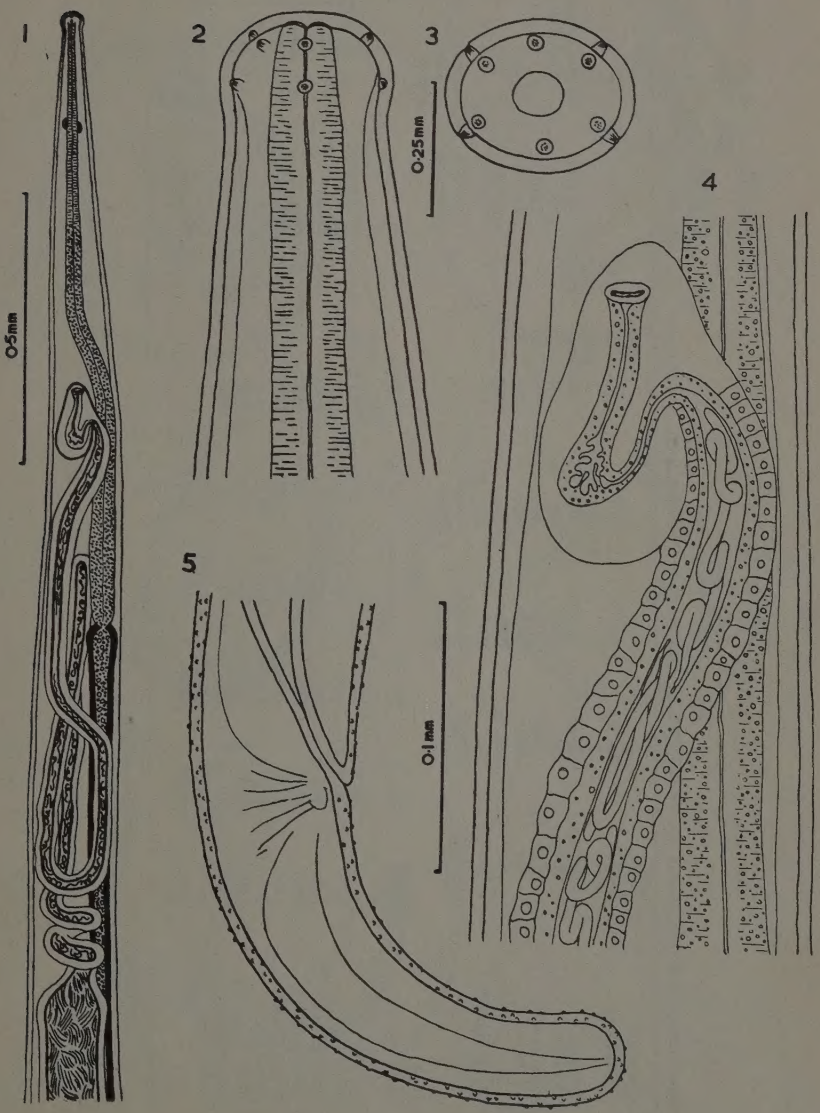
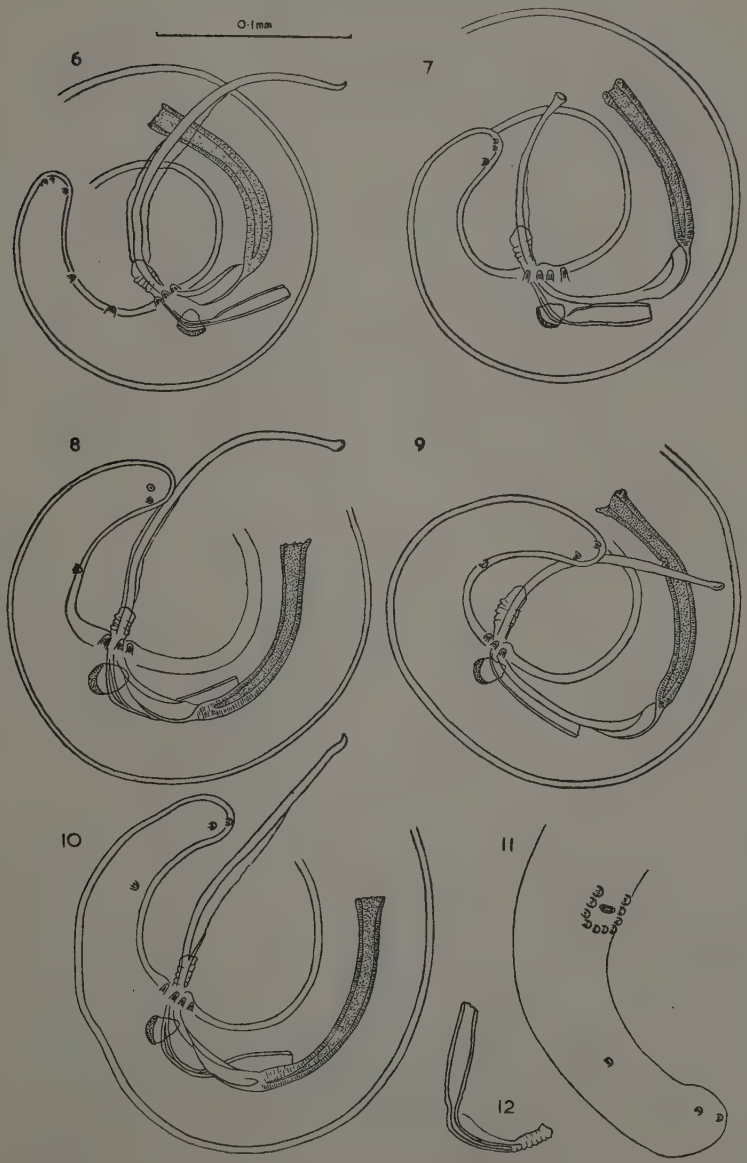

1
0·5mm
2
3
0·25mm
4
5
0·1mm

TABLE I

Measurements of *Wuchereria* sp. (*malayi*?) from Kra monkey (*Macaca irus*) in Pahang.

						Measurements of *W. malayi* from Man	
						India (Rao & Maplestone)	Indonesia (Bonne *et al.*)
FEMALE							
Specimen	No. 1	No. 2	No. 3	No. 4 (immature)	Mean (Nos. 1, 2, 3)		
Length	52 mm.	43.5 mm.	49 mm.	25 mm.	48.2 mm.	55 mm.	40 mm.(imm.)
Breadth	170	145	130	70	148	160	120–170
Oesophagus ...	1120	1050	1050	820	1073	1380	—
*Vulva	760	680	630	520	690	980	900
Tail	190	230	135	130	185	940(?)	280
†Head diam. ...	38	35	36	28	36		—
Habitat	‡Lymph vessel	Lymph vessel	Lymph vessel	Abdominal gland		Cyst on forearm	Inguinal and popliteal glands
MALE							
Specimen	No. 1	No. 2	No. 3	No. 4	No. 5	Mean (Nos. 1–5)	
Length	18.5 mm.	23.3 mm.	13.5 mm.	20.5 mm.	16 mm.	18.3 mm.	22–23 mm.	26 mm.
Breadth	75	80	75	75	70	75	88	90
Oesophagus ...	900	980	960	1000	880	944	1120	—
Left spicule ...	340	355	365	360	290	342	350	360
Right spicule	100	110	100	120	100	106	115	120
Spic. ratio... ...	3.3 : 1	3.3 : 1	3.8 : 1	3.3 : 1	2.9 : 1	3.3 : 1	3 : 1	3 : 1
Tail	140	150	150	140	132	142	100–140	150
Head diam. ...	28	30	28	30	23	28	—	—
Habitat	Lymph vessel	Lymph vessel	Lymph vessel	Lymph vessel	Abdominal gland		Cyst on forearm	Inguinal and popliteal glands

* Distance from anterior extremity.
† Measured dorso-ventrally.
‡ The lymph vessel from which 7 worms were recovered, was in close proximity to the spermatic cord.
(All measurements in μ except where otherwise stated.)



Wuchereria sp. (*malayi*?) from *Macaca irus*.

Figs. 6 to 10.—Tails of five different male specimens, left lateral view. Fig. 11.—Tail of male specimen, sub-ventral view, showing number and arrangement of adanal papillae. Fig. 12.—Right spicule, seen from right-hand side. (For the sake of clarity, the pair of posterior adanal papillae have been omitted from Figs. 6 to 10.)

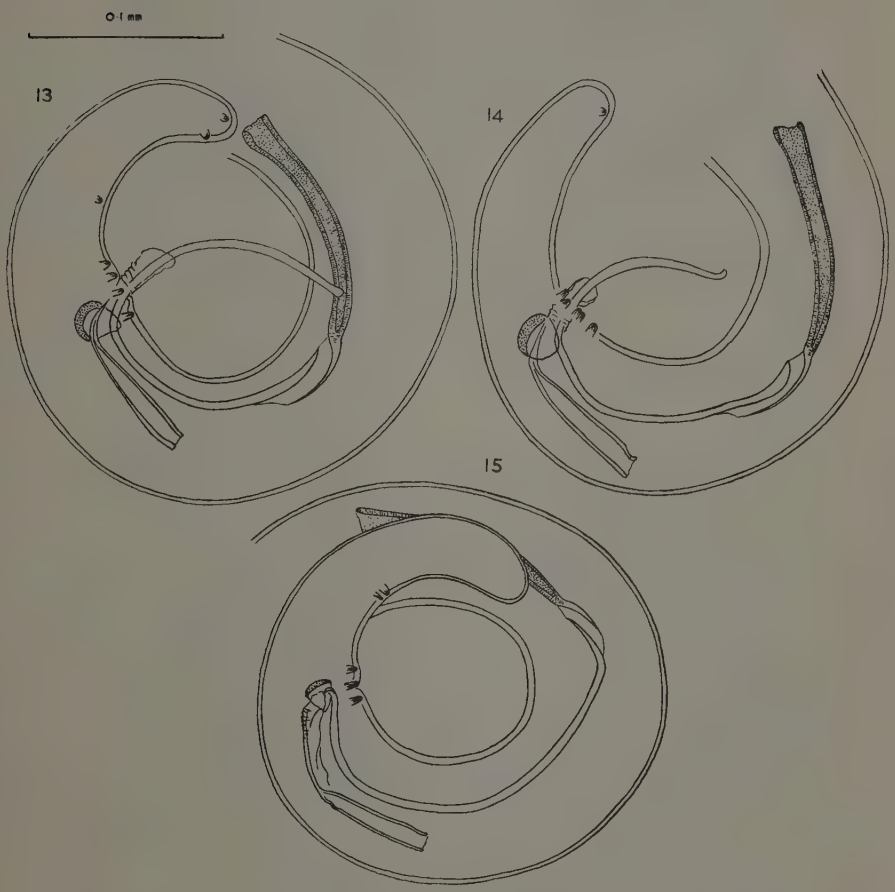
spicule just described. The free part of the "leaf" is curled and at its distal extremity it runs into the third or distal part of the spicule. This is a solid rod of almost uniform thickness which in all 5 specimens was extruded through the ano-genital aperture for most of its length, outside the body of the worm. After a very slight narrowing near the extremity, it terminates in a characteristic spatulate tip, as in the left spicule of *W. bancrofti*. In 4 of the specimens (Figs. 6, 7, 8 and 10) a very delicate membrane or sheath was seen attached to it for most of its length. In one specimen (Fig. 10) the membrane appeared to have origin at a sharply pointed process arising from the spicule near its base.

The right spicule is characteristically curved ventrally, to a greater or less degree. Its proximal half is tubular and is open at its proximal extremity. This tube narrows abruptly to form the distal half which terminates in a short cylindrical part with a corrugated surface, through which the left spicule runs. Its extreme tip is uncorrugated. The gubernaculum is very similar to that in *W. bancrofti*.

Wuchereria sp. (*malayi*?) from Domestic Cats

Adult worms or fragments were recovered from 3 cats; their measurements are seen in Table II. The two males from Cat No. II were of especial interest in respect to the unusual circumstances in which they were found. A sub-spherical cyst of 6—7 mm. in greatest diameter was observed in conjunction with a popliteal gland and when it was removed and examined under light pressure in a compressorium it was seen to be a thin-walled transparent sac containing straw-coloured liquid in which there were worms in violently active motion. These proved later to be two male *Wuchereria* attached by their head ends to the cyst wall, but when first seen their movement was so rapid that they could not be counted nor could their sex be determined. This occurrence was in contrast with all the other adults which were recovered from gland tissue or vessels and were sluggish in their movements. Microfilarial counts made every two hours for 24 hours on Cats I and VI showed considerable numbers during the day although the numbers increased at night.

The tail region of the two males for Cat No. II are illustrated in Figs. 13 and 14 and that of the single male from a cervical gland in Cat No. VI is seen in Fig. 15. These figures are drawn to the same scale as Figs. 6—10 from the Kra monkey and the generally



Wuchereria sp. (*malayi*?) from domestic cat.

Figs. 13 and 14.—Tails of two different male specimens, left lateral view, from popliteal gland, Cat No. II. Fig. 15.—Tail of male specimen from cervical gland, Cat No. VI. (Posterior pair of adanal papillae omitted.) N.B.—These drawings are to the same scale as Figs. 6–11.

TABLE II

	Measurements of *Wuchereria* sp. (*malayi?*) from Cats				Measurements (mean) of *Wuchereria* sp. (*malayi?*) from Kra monkey.
FEMALE	Cat VI	Cat II	Cat II	Cat I	
Length	—	—	—	—	48.2 mm.
Breadth ...	135	—	—	—	148
Oesophagus ...	1400	—	—	956	1073
Vulva	640	—	—	500	690
Tail	—	—	—	—	185
MALE					
Length	—	19.5 mm.	25.2 mm.	—	18.3 mm.
Breadth ...	75	82	82	—	75
Oesophagus ...	950	850	900	—	944
Left spicule ...	380	410	390	—	342
Right spicule	110	137	122	—	106
Spicule ratio...	3.4 : 1	3 : 1	2.9 : 1	—	3.3 : 1
Tail	150	170	140	—	142
Habitat... ...	Cervical gland	Cyst on popliteal gland	Cyst on popliteal gland	Cervical gland	Lymph vessel

(All measurements in μ except where otherwise stated.)

larger dimensions, especially of the left spicules, of the specimens from the cats are notable. There is no difference, however, in the morphology of the spicules in the specimens from the cat and those from the monkey and although there are fewer terminal papillae (in Fig. 15 no terminal papillae could be seen) this can hardly be regarded as significant in such a small number of specimens. The delicate membrane or sheath was not observed on the spicule. Female material is very scanty ; the fragment from Cat No. VI has a long oesophagus (1400μ) compared with that in the females from the monkey, (average 1073μ). The position of the vulva is more anterior in relation to the oesophagus, being anterior to the half-way mark, whereas in the monkey material the vulva is posterior to this point. The female fragment from Cat No. I was probably immature and its measurements are probably not representative.

The possible taxonomic significance of these dimensional differences in *Wuchereria* from the cats cannot be assessed until more abundant material is available.

Morphological Relationships of *Wuchereria* sp. from Monkey and *W. malayi* from Man

Comparison of the measurements of the specimens from the monkey with those published by Rao and Maplestone (1940) and Bonne *et al.* (1941) shows a fairly close agreement between the specimens from man and those from the monkey, with the exception of the length of the tail of the female, given by Rao and Maplestone as 940μ, which must be an error (Table I). Comparison, however, of the descriptions and illustrations of the Indian and Indonesian *W. malayi* with the present specimens shows a lack of agreement in certain respects which must be considered and assessed as far as possible before a specific diagnosis of the monkey specimens can be attempted. This lack of agreement is concerned with spicules and caudal papillae in the male.

Taking first the illustrations of this region by Rao and Maplestone, in both Figs. 1 and 2 the longer left spicule is depicted as being in two parts, a proximal broad part and a distal narrow part about twice as long, i.e. about $\frac{2}{3}$ the total length. The broader proximal part is smooth (i.e. without granular stippling) and the curled leaf-like portion, so characteristic of the left spicule of the monkey specimens is not depicted. However, "the tip shows a small membranous spoon-like expansion" which corresponds with the spatulate

tip in the monkey specimens. The description and illustrations by Rao and Maplestone of the short right spicule are not sufficiently detailed to allow of a fair comparison to be made between them and the right spicule of the specimens from the monkey.

Regarding the caudal papillae they mention "two pairs of large papillae one immediately in front of and the other just behind the cloaca and in close apposition to them there are two pairs of smaller papillae. No other papillae could be observed". Although numerically in agreement with these papillae in the monkey specimens, the grouping described (which is also apparent in their Fig. 1) and the distinction between large and small papillae are not the same. (Their failure to observe any other papillae may be assumed to be an oversight.) In view of these apparent differences in spicular morphology and in the arrangement and size of papillae in the male, it is considered impossible to conclude that the *Wuchereria* from the monkey is the same species as that described by Rao and Maplestone.

Turning now to the description and illustrations given by Bonne *et al.* of their one complete male worm, the two photographs (Figs. 5a and 5b) are extremely valuable and show a striking resemblance to the tails of the male specimens from the monkey. The extremity of the shorter (right) spicule, extruded through the cloaca has exactly the same appearance as that in the monkey specimens; it is furnished with annular rugae and terminates in a plain non-annulated tip. The left spicule is said to be "not much flattened or widened" at the tip. In their Fig. 5a the tip seems to be pointed and slightly curved, but this is exactly what is seen when the spatulate tip of the monkey specimens is seen in lateral view, as in Figs. 6 and 10 of the present paper. It is significant too that "an extremely delicate membrane follows the free part of the spicule for over half its length", which conforms with that described in the specimens from the monkey. The line drawing of the male tail is not very helpful as too much emphasis has been given, at the expense of more important spicular detail, to "a sheath with oblique opening and spiral strengthening of its wall", and no mention is made of the twisted intermediate portion. It is probable, however, that this portion of the spicule was overlooked by Bonne *et al.* and perhaps also by Rao and Maplestone. Bonne *et al.* observed four thick papillae on the left side and three, possibly four, on the right. They also mention "probably two tiny postanal submedian papillae on each side, one very near the tip of the tail and one about halfway". They do not mention any grouping of the adanal papillae as noted by Rao and Maplestone nor distinction between small and large papillae.

It is evident that the *Wuchereria* sp. from the Kra monkey in Pahang is very close to and probably identical with the species from man described by Bonne *et al.* and a re-examination of their material would quickly put the matter beyond doubt. The status of the specimens of Rao and Maplestone is now somewhat uncertain in view of the discrepancies between their description and that of Bonne *et al.* Whether these discrepancies are significant or not can only be resolved by a re-examination of both lots of material from man and it is most important that this should be carried out so that a taxonomical analysis of the problem can be attempted. The fact that the microfilariae are apparently identical is no longer a valid criterion from which to draw conclusions about the identity of *Wuchereria* spp. of the *"malayi"* type, since a clearly distinct species has been discovered in a dog and a cat (present paper) whose microfilariae are apparently identical with those from monkey and from man in Malaya and elsewhere.

Wuchereria pahangi n.sp. from a Dog and a Cat

A mongrel bitch whose blood contained *malayi*-type microfilariae was sacrificed at Kuantan on the 1st August, 1955, and its lymphatic glands were removed and examined in the manner described earlier. Fragments of a nematode worm were found near a popliteal gland which under the compressorium was seen to contain calcified remains of a nematode. Later from the axillary glands 3 intact male specimens and parts of a female were obtained. The male worms measured 12·6, 15·9 and 17·3 mm. respectively and the parts of the female which included head and tail regions, made a total length of 40 mm. Further measurements of these specimens are seen in Table III, and they are illustrated in Figs. 16 to 22.

A domestic cat (No. III) with *malayi*-type microfilariae in its blood was sacrificed on 26th August at Kuantan and search for adult worms was made in the lymphatic system. One male 14·5 mm. long and parts of a female totalling 28 mm. were obtained from a popliteal gland. The female was found with its anterior end embedded in the gland and the male was free in the dish of saline in which the glands were awaiting examination. These specimens proved to be the same species as that from the dog. The chief measurements are seen in Table III and the male tail is illustrated in Figs. 23 and 24. The microfilarial count in this cat was low and showed a periodicity similar to that found in Cats I and VI.

Morphology

A perusal of the table of general dimensions shows that these worms are smaller than those from the monkey (No. IV), cats (Nos. I, II and VI) and those described from man. The body cuticle is unstriated throughout (except in the ventral surface of the male tail) and the bulbous head and head papillae are of the *Wuchereria* type. The oesophagus is more clearly divisible into two parts in both sexes than in the material previously described in this paper. The anterior muscular part is thinner than the glandular part and the junction of the two can easily be seen (Fig. 16). It is probable that this is a character of value in specific diagnosis.

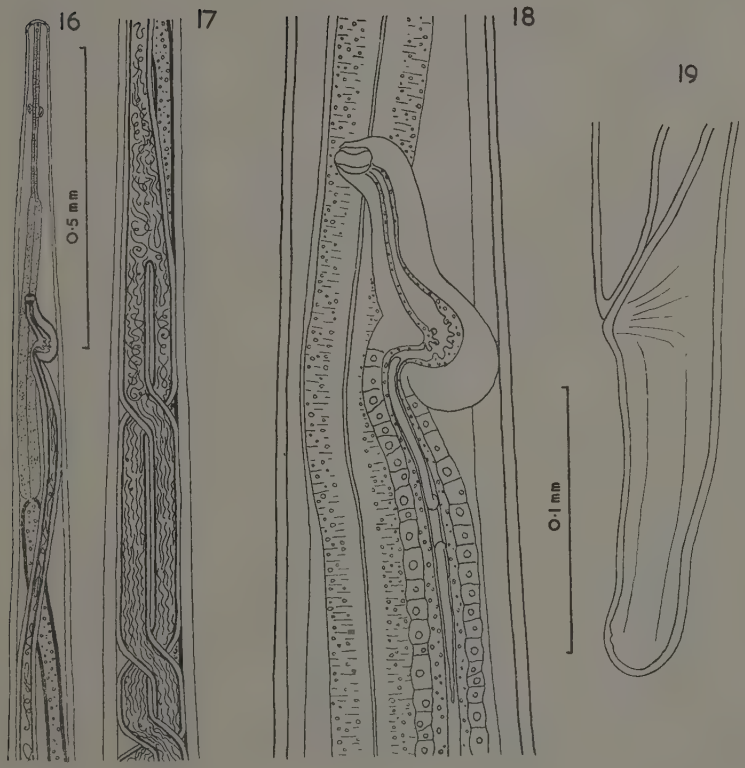
The female

The *ovejector* and *vagina* have the same structure as in the previously described material but in the specimens examined the vagina was uncoiled and after joining the uterus the latter shortly bifurcates into its two branches. The position of the vulva in relation to the oesophagus was in both specimens behind the mid-point of the latter. The tail of the female has the usual *Wuchereria* shape but in the two specimens available for examination the cuticle is entirely devoid of the minute cuticular bosses which occur in *W. bancrofti* and in *Wuchereria* sp. (*malayi*?) from the monkey. The absence of these bosses may be regarded as a character of value in specific diagnosis and should be of use where only fragments of worms are recoverable.

The male

The tail region has the same general pattern as that of the specimens from monkey and cats (above) but differs markedly in the length of the tail, the spicules and in the spicule ratio. (See Table III). A very marked structural difference, however, is seen in the left spicule whose distal part is much shorter and somewhat thinner than that of the previous species ; moreover, it lacks the spatulate termination so characteristic of the previous species and of *W. bancrofti*. No delicate membrane was seen on the distal part of this spicule, but in the retracted spicule it would be difficult to see.

The papillae do not differ much in their number and arrangement. The lateral adanals vary from 3 to 5 on either side and the pair of



Wuchereria pahangi n.sp. from dog.
Figs. 16 and 17.—Anterior portion of female. Fig. 18.—Vulva, ovejector and part of vagina. Fig. 19.—Tail of female. (Note the smooth cuticle.)

posterior adanals is present. The intermediate post anals and the terminal papillae are also characteristically variable in number and position.

This species is clearly different from all the other known species of *Wuchereria* and is named *W. pahangi* n.sp. after the locality in which it was first discovered.

TABLE III

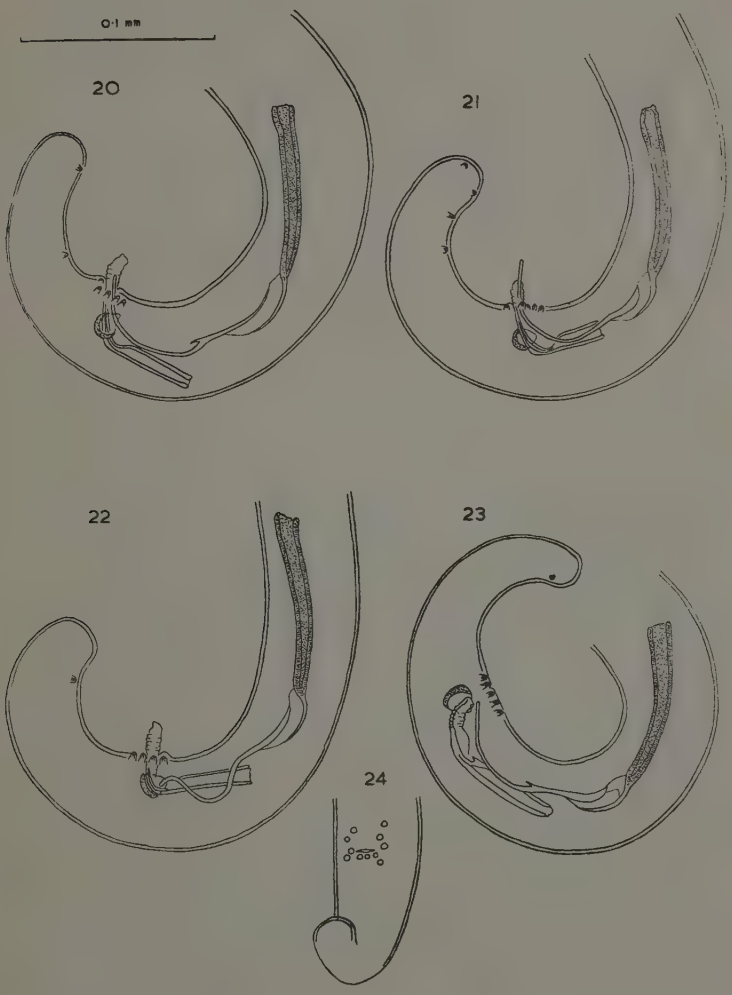
Measurements of *Wuchereria pahangi* n.sp. from Dog and Cat.

Host	Dog I	Dog I	Dog I	Cat III
FEMALE				
Length	40 mm.			28 mm. (incomplete)
Breadth	100			100
Oesophagus	755			880
Vulva	450			490
Tail	140			155
Head diam.	36			
MALE				
Specimen No. ...	No. 1	No. 2	No. 3	
Length	12.6 mm.	15.9 mm.	17.3 mm.	14.5 mm.
Breadth	75	80	75	70
Oesophagus	820	930	920	740
Left spicule	210	208	215	200
Right spicule ...	85	75	90	83
Spicule ratio... ...	2.5 : 1	2.8 : 1	2.4 : 1	2.4 : 1
Tail	110	130	125	120
Head diam.	23	26	24	
Habitat...	Axillary gland	Axillary gland	Axillary gland	Popliteal gland

(All measurements in μ except where otherwise stated.)

Wuchereria pahangi n.sp. from dog and cat.

Figs. 20 to 22.—Tails of three different male specimens, left lateral view, from axillary gland, Dog No. I. Fig. 25.—Tail of male from popliteal gland, Cat No. III. Fig. 24.—Tail of male, ventral view of Fig. 23, showing number and arrangement of adanal papillae. (One posterior adanal papilla is illustrated in Fig. 20.) (In Fig. 21 the distal part of the left spicule *appears* to run through the tubular proximal part of the right spicule. Actually, it is only superimposed upon it.)


0·1 mm
20
21
22
23
24

Hosts : Domestic dog, domestic cat and (?) Slow Loris.

Habitat : Lymphatic system.

Locality : Pahang, Malaya.

Types : In the Department of Parasitology, London School of Hygiene and Tropical Medicine.

The Slow Loris (*Nycticebus coucang*) is included tentatively in the above list of hosts of the new species because of fragments of a female *Wuchereria* which were recovered on one occasion from the abdominal glands of this animal. The fragments included the posterior extremity only ; the cuticle in the tail region was perfectly smooth, without cuticular bosses, and thus conforms with *W. pahangi* in at least one character.

Discussion

The recovery for the first time from non-human hosts of adult specimens of *Wuchereria*, comprising at least two distinct species of this important genus, has increased the number of problems, taxonomical, biological and epidemiological which automatically followed the discovery of *malayi*-like microfilariae in the blood of Kra monkeys in 1939 by Poynton & Hodgkin, subsequently confirmed by the staff of the Filariasis Research Laboratory at Kuantan who discovered four new kinds of hosts for these microfilariae, two primates and two carnivores.

The correct identification of the adult forms of these microfilariae, which are apparently identical in this larval form in all the hosts, is clearly a most desirable end in itself but from the epidemiological aspect it is ancillary to the problem of the possible rôle of these animals as reservoirs of the human infection. Should, for example, the adult worms from the Kra monkey prove to be identical with those harboured by man, it cannot be assumed from this morphological evidence that reciprocal infection by this species between the two kinds of hosts is a physiological possibility. (c/f *Ascaris lumbricoides* in man and pig.) Cross-transmission experiments alone can provide the answer to this problem.

The new knowledge that in Malaya there are more than one species of *Wuchereria* raises two most intriguing questions : Which species is harboured by man or can he, like the cat, harbour more

than one species? As pointed out by Jachowski (1955): "Basically, the parasite concerned has not been positively identified. The human filaria is probably *W. malayi*, but this identification is based solely upon microfilarial morphology and known biological properties of this species. Adult worms recovered from human hosts by biopsy or autopsy should be studied. However, religious customs (Moslem) of the Malays, make such studies difficult, if not impossible."

Fortunately, there is now at hand a possible alternative method to human biopsy or autopsy for the recovery of these adult worms, namely transmission of the human filarial infection *via* mosquitoes to an easily available and perhaps easily infectible laboratory animal, the domestic cat.

Summary

(1) Adult specimens or parts of specimens of *Wuchereria* were recovered from the lymphatic systems of a Kra monkey (*Macaca irus*) (1), domestic cats (4), a dog (1) and a Slow Loris (*N. coucang*), from areas of endemic human filariasis in Pahang, Malaya.

(2) Morphological studies on this material revealed a new species, herein named *W. pahangi*, in the dog and in one cat; a species in the Kra monkey which is close to and probably identical with adults of *W. malayi* as described by Bonne *et al.* (1941) from man in Indonesia; but its relationships, and also those of the species from man in Indonesia, with the *W. malayi* described by Rao & Maplestone (1939) in India need further investigation. Three cats harboured a species which is close to that in the Kra monkey but its identity is *sub judice* until further material is available. Fragments only of a female worm, which included the tail, were recovered from the Slow Loris. It is thought that this material may be the new species *W. pahangi*.

References

Bonne, C., Lie Kian Joe, Molenkamp, W. J. J. and Mreyen, F. W., 1941.—"*Wuchereria malayi*, de macrofilaria behoorende bij de microfilaria malayi." *Geneesk. Tijdschr. Ned.-Ind.*, **81**, 1487–1501. (W.L. 9002.)

Buckley, J. J. C., 1952.—"Filariasis in Fiji: addendum." *Trans. R. Soc. trop. Med. Hyg.*, **46**, 321–324. (W.L. 21671.)

——1952a.—"Demonstration of cuticular bosses in *Wuchereria bancrofti*." *ibid.*, (demonstration), **46**, 374.

Edeson, J. F. B., Wharton, R. H. and Buckley, J. J. C., 1955.—"Filarial parasites resembling *Wuchereria malayi* in domestic and forest animals in Malaya." *ibid.*, (correspondence), **49**, 604–605.

EDESON, J. F. B., WHARTON, R. H., and BUCKLEY, J. J. C., 1956.—"Adult specimens of *Wuchereria* recovered from forest and domestic animals in Malaya. *ibid.*, (demonstration), **50,** 1.

JACHOWSKI, L. A., 1955.—"A review of research on filariasis in Malaya." *Lecture and review series. National Medical Research Institute, National Naval Medical Center, Bethesda, Maryland.* No. 55–57, 345–359.

POYNTON, J. O. and HODGKIN, E. P., 1939.—"Two microfilariae of the Kra monkey, (*Macaca irus*)." *Trans. R. Soc. trop. Med. Hyg.*, **32,** 555–556.

RAO, S. and MAPLESTONE, P. A., 1940.—"The adult of *Microfilaria malayi* Brug, 1927." *Indian med. Gaz.*, **3,** 159–160. (W.L. 9943.)

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A Note on the Specific Identity of *Trichostrongylus longispicularis* Gordon, 1933

By R. I. Sommerville, M.Agr.Sc.
McMaster Laboratory, C.S.I.R.O., Sydney, N.S.W.

The nematode *Trichostrongylus longispicularis* was described by Gordon (1933) from a single male recovered from a sheep in New South Wales. Gordon considered that the male of this species could be readily distinguished from the males of other species of the genus recorded from ruminants by an asymmetrical dorsal ray of the bursa and by the length and form of the spicules. The dorsal ray is described as being bifid, one bifurcation being simple and the other possessing secondary branches, one situated internally and the other externally. The slender spicules were 184·6 microns long, and terminated in fine sickle-shaped structures.

Andrews (1934 and 1935) recorded the species from cattle in the United States. In his first description (Andrews, 1934) he noted that his specimens agreed very closely with the description published by Gordon (1933), but he referred to hook-like projections on the spicules. However, he failed to find these projections in the specimen discussed in his record of 1935. As he made no reference to the dorsal ray of the bursa, it is presumed that this agreed with the description and figure published by Gordon (1933). *T. longispicularis* was subsequently reported by Roberts (1938 and 1939) from cattle in Queensland, but no comments were made on its morphology.

Some doubt has been thrown on the validity of the species by LeRoux (1950), who reported the recovery from sheep in the Cape Province of South Africa, of specimens considered to be *T. colubriformis* (Giles, 1892), in which the spicules measured up to 190 microns in length and were similar in form to those described by Gordon (1933) for *T. longispicularis.*

Recently some specimens of a species of *Trichostrongylus* recovered from a calf in Western Australia were sent to the McMaster Laboratory and were identified by Mr. Gordon as *T. longispicularis.* An opportunity was thus afforded of comparing these specimens with specimens of *T. colubriformis.*

The females of the material from Western Australia could not be distinguished from those of specimens of *T. colubriformis.* The structure of the male bursa was identical with that of *T. colubriformis* and the dorsal ray did not display the asymmetry recorded by Gordon for this species. The proximal portions of the spicules of

both species were similar. Distally however, the prominent barb of *T. colubriformis* was represented in *T. longispicularis* by a minute projection, visible in lateral view, as described and figured by Andrews (1934) and illustrated in Figs. 3 and 4. The spicules as a whole were much darker than those of *T. colubriformis* and were also broader. The mean lengths of both right and left spicules of *T. longispicularis* (Table 1) were significantly greater ($p < \cdot001$) than the mean lengths of both right and left spicules of *T. colubriformis* (Table 1). Measurements of the spicule length of *T. colubriformis* in Table 1 were taken from Nagaty (1933) and the largest spicule measured did not exceed 170 microns. However, LeRoux (1950) has shown that *T. colubriformis* spicules may be as long as 190 microns, so that there is some overlapping between the two species.

The tips of the spicules of *T. longispicularis* were markedly different from those of *T. colubuformis*. In the latter species the right spicule was bluntly rounded, whereas the left terminated in a point (Figs. 5 and 6). In *T. longispicularis* both spicules were blunt and rounded at the tips (Figs. 3 and 4) and a tongue-like semi-transparent membrane projected from the tip of the spicule proper. In the right spicule (Fig. 3) this was displaced ventrally, whereas in the left spicule (Fig. 4) it continued along the central axis without displacement. This structure was entirely lacking from the tips of spicules of *T. colubriformis*.

The gubernaculum of *T. longispicularis* was significantly longer ($p < \cdot01$) than in *T. colubriformis* and as can be seen from Figs. 1 and 2, the shape and structure was also different.

Measurements of the total length of male and female *T. colubriformis* and *T. longispicularis* are shown in Table 1, together with measurements of the spicules of the males.

In addition to morphological features, *T. longispicularis* is further distinguished from *T. colubriformis* by its occurrence in cattle rather than in sheep. Thus, in three studies of parasitism in cattle, it has been noted to occur (Andrews 1934 and 1935 ; Petersen, personal communication ; Roberts 1938 and 1939), but there is only one record in the literature of its recovery from sheep and then as an isolated specimen (Gordon, 1933).

Roberts (personal communication) found that 33 of 178 Queensland cattle were infested with *T. longispicularis*, in numbers which ranged from 15 to 220 worms. However, only 4 animals in the same group were found to be infested with *T. colubriformis*. Petersen (personal communication) recovered *T. longispicularis* from both calves and yearlings in the Busselton—Margaret River area of

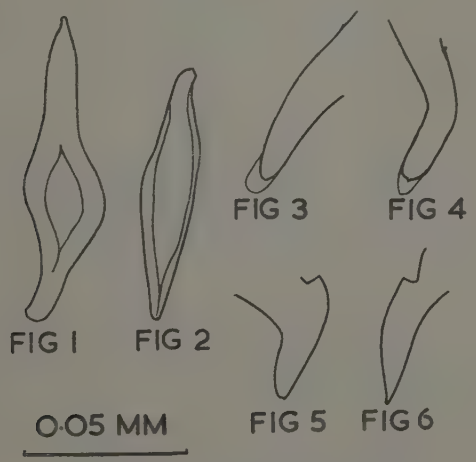
TABLE I

Measurements in (microns except where otherwise stated) of *T. longispicularis* and *T. colubriformis*

	T. longispicularis		*T. colubriformis*	
	Range	Mean	Range	Mean
Total length (mms.)				
Males	5·7—7·5	6·2	4·3—7·7	5·5
Females	5·2—9·0	7·9	5·0—8·6	6·6
Length left spciule ...	180—200	186	132—167	148
Length right spciule ...	168—187	176	123—154	135
Distance from barb to tip				
Left spicule ...	43—46	44	35—41	36
Right spicule ...	45—53	49	30—41	35
Length of gubernaculum ...	85—110	94	61—83	72

Measurements of *T. colubriformis* are from Nagaty (1933).

Measurements of total length and of spicules of male *T. longispicularis* include data from Gordon (1933) and Andrews (1935).



T. longispicularis and *T. colubriformis*

Figure 1. Gubernaculum of *T. longispicularis*.

Figure 2. Gubernaculum of *T. colubriformis*.

Figures 3 and 4. *T. longispicularis*. Distal end of right and left spicule respectively.

Figures 5 and 6. *T. colubriformis*. Distal end of right and left spicule respectively.

Western Australia. It was the only species of *Trichostrongylus* present in the small intestines of the animals examined, although *T. axei* was present in the abomasum. *T. longispicularis* was present in the majority of calves examined, the highest infestation recorded being 2,000 worms. It is therefore not uncommon in cattle in certain regions of Australia and can occur as a pure infestation.

Apparently *T. longispicularis* is somewhat variable in the morphology of the bursa and spicules. Sometimes, as in the specimens described by Gordon (1933), and presumably also in those seen by Andrews (1934 and 1935), the dorsal ray is asymmetrical, whereas in the specimens from Western Australia it is similar to that of *T. colubriformis*. Hooks on the spicules may be present or absent, but when present they are never as strongly developed as they are normally in *T. colubriformis*. Morphological variants of *T. colubriformis* are also known (Monnig, 1933) but their occurrence is sporadic. Furthermore, there is no evidence of pure infestations of these variants, for they appear to occur in association with the normal type.

It is considered therefore, that the evidence is sufficient to establish the validity of the species *T. longispicularis*, the description of the spicules and gubernaculum given above being sufficient for identification.

REFERENCES

Andrews, J. S., 1934.—"*Trichostrongylus longispicularis* collected from cattle in United States". *Proc. Helminth. Soc. Wash.*, **1**, 13. (W.L. 16747a).

———1935.—"A second report of the occurrence of *Trichostrongylus longispicularis* in cattle in the United States". *ibidem*, **2**, 90.

Gordon, H. McL., 1933.—"Some ovine Trichostrongylids reported from Australia for the first time, with a description of *Trichostrongylus longispicularis* sp.nov., from a sheep". *Aust. Vet. J.*, **9**, 34–37. (W.L. 2254).

Le Roux, P. L., 1950.—"*Trichostrongylus leiperi* sp.nov., a parasite of the Eland (*Taurotragus oryx*) in Northern Rhodesia." *J. Helminth.*, **24**, 23–27. (W.L. 11224b).

Mönnig, H. O., 1933.—"Wild antelopes as carriers of nematode parasites of domestic ruminants. Part III". *Onderstepoort J. Vet. Sci.*, **1**, 77–92. (W.L. 15681a).

Nagaty, H. F., 1932.—"The genus *Trichostrongylus* Looss, 1905". *Ann. Trop. Med. Parasit.*, **26**, 457–518. (W.L. 1063).

Roberts, F. H. S., 1938.—"The gastro-intestinal helminths of cattle in Queensland : their distribution and pathogenic importance". *Proc. Roy. Soc. Qd.*, **50**, 46–54. (W.L. 16903).

———1939.—"The occurrence and prevalence of gastro-intestinal helminths in apparently healthy cattle in Queensland, Australia". *J. Comp. Path.*, **52**, 160–165. (W.L. 11136).

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Studies of *Litomosoides carinii* by Phase-contrast microscopy: the Development of the Larvae

By James A. McFadzean and J. Smiles

National Institute for Medical Research, London

The introduction of *Litomosoides carinii* infection in cotton rats in the laboratory for screening drugs for antifilarial activity (Culbertson & Rose, 1944) constituted a major advance in the experimental study of filariasis. There is, however, little known about the development of the microfilariae. Stained preparations, and living material examined by ordinary microscopical techniques reveal little structural detail. It has been shown, however, in mammalian ova, which are normally too large for examination by phase-contrast microscopy, that detailed structure may be revealed by this method when the cells are flattened between slide and coverglass (Austin & Smiles, 1948). Preliminary observations on living microfilariae prepared by a modification of the above technique showed that phase-contrast microscopy would be a useful method of investigating the development of the microfilariae.

Material and Methods

Litomosoides carinii infection in cotton rats is maintained at the National Institute for Medical Research by the method described by Hawking and Sewell (1948). Infected cotton rats were killed by coal gas. The skin and pectoral muscles were reflected from the chest wall, the thorax opened and the adult worms removed from the pleural cavity and washed in Ringer's solution to remove the cells and debris of the pleural fluid. An adult female worm was stretched out in Ringer's solution on a glass slide and immobilised by placing small metal weights on the head and on the tail. Under a dissecting microscope the worm was incised at intervals along its length with a dissecting needle and the contents of the uterus were collected in fine capillary pipettes as they emerged from each incision.

There was no necessity to dissect out the uterus. By this method large numbers of larvae at different stages of development were obtained. The technique of preparing the specimens for examination was as follows. A thin layer of melted agar was poured on a glass slide and when set, a drop of the suspension of the larvae from a pipette was placed on the agar. A coverglass was gently lowered on to the drop and in the case of the earlier larvae, the weight of the coverglass was sufficient to flatten the cells to obtain adequate representation of the structures in the magnified microscopical image. The technique of using agar has the advantages of the agar acting as a soft pad and preventing rupture of the larvae, and partially immobilising actively moving forms. The degree of flattening of the specimen can be controlled by varying the strength of the agar and by using different weights of coverglasses. In these investigations 1 per cent agar in Ringer's solution was used in conjunction with a No. 2 coverglass ($\frac{7}{8}'' \times \frac{7}{8}''$). From the observation that specimens of red blood corpuscles prepared in this way do not undergo any change over a considerable period, it is possible to exclude any morphological changes occurring in the larvae due to contact with the agar. Photomicrographs were taken of the different stages and a series depicting the development is here reproduced.

Results

The early forms, due to their soft consistency, were easily flattened by the weight of the coverglass. Fig. 1 shows an ovum which has been flattened to show detail in the nucleus and cytoplasm. The oval nucleus lies parallel to the long axis of the cell and contains diffuse nuclear material in clear nuclear sap. The cytoplasm contains large dark granules towards the periphery of the cell, between them and the nucleus are short filamentous structures, and immediately round the nucleus is a narrow zone of densely packed granules.

The daughter cells of the first cleavage are always unequal in size (Fig. 2). The nuclei are spherical and lie towards the opposite poles of the cells. The cytoplasmic organellae are similar to the above except that in the great majority of specimens the large granules are concentrated in one of the cells.

Each subsequent cleavage division results in a reduction in the size of the cells and in many cells there is a decrease in the volume of the cytoplasm relative to that of the nucleus (Figs. 3-7). In the unflattened cells, the external membrane can be clearly seen enclosing the "vitelline" space and the embryonic cells, but due to the depth of the cell and the large number of cytoplasmic organellae, the outlines of the nuclei are never clearly depicted (Figs. 3 and 4).

When flattened, the cells occupy the whole of the "vitelline" space (Figs. 5 and 6). The structures within the cells, especially the nuclei and nucleoli, are then more clearly seen.

Organisation of the developing larvae is seen first in the appearance of large vacuole-like bodies of low refractive index each containing a small highly refractile irregularly shaped structure. Each of these bodies, usually three or four in number, resembles the excretory cell seen in more mature larvae. About the same time two or three large dark granules appear, scattered in the structure of the larva. These are illustrated in Fig. 7. Subsequently the larva begins to take shape as shown by an indentation, seen in Fig. 8 on the right side of the larva. The vacuoles noted in the previous figure are still present. There is an increase in the numbers of large dark bodies, usually to four or five but there may be many more. The head and tail of the larva are now differentiated as a broad and a narrow end of the cellular structure (Figs. 9 and 10). At the same time the first sign of a hook like structure is seen on the head end of the larva.

The next stage in development is the formation of a body cavity in the larva which commences dorsally near the tail. This subsequently develops until it occupies the greater part of the body of the larva. (Figs. 11 to 15). During the formation of the body cavity, the larva increases in length and becomes a much slimmer structure. The formation of the cavity appears to be due to a reorganisation of the original cells, associated with the formation of a subcuticular strand of highly refractile cells. The latter appear as the body cavity is being formed and are shown on the dorsal surface near the tail in Fig. 11. This process continues until the larva appears as a hollow shell with an outer framework of the highly refractile subcuticular cells in which no structural detail can be seen. When the process is complete (Fig. 15) corrugations are visible on the surface of the larva. These subsequently become more distinct and more delicate. The hook structure also gradually develops as shown in Figs. 11 and 13. Movement of the larva is first noted at the stage of development shown in Fig. 11 and becomes more marked as the larva matures.

Further development is characterised by the body cavity becoming filled in with other cells, commencing at the head and extending towards the tail until the cavity is completely filled (Figs. 16-18). These cells have a lower refractive index than the subcuticular cells and appear to be of several types. The subcuticular cells themselves gradually become more rounded and are seen to be

connected to one another by strands of cytoplasm. (Fig. 17). Later they diminish in size.

From the stage shown in Fig. 16 the larva is actively moving within the external membrane. Eventually it succeeds in stretching the membrane. This stretching takes a considerable time and the process has been observed for several hours, during which the larva made only slight progress. The sequence of events is shown in Figs. 19 to 22. A few specimens were observed where the membrane had been elongated, and where the larva had also made a pocket on one side. The excretory pore and excretory cell became clearly visible as the larva extended. (Fig. 21). In Fig. 21 the excretory pore is surrounded by a dark refractile area, which in turn is surrounded by a clear area. This arrangement is only seen in a proportion of the larvae, and may be related to the functioning of the excretory apparatus.

Finally, the larva becomes fully extended within the external membrane which now becomes the "sheath" of the microfilaria (Fig. 22) and which at first is rounded at both ends. Later, the tail end becomes tapered due to the movement of the larva to and fro within the sheath.

In the fully extended larva, three constant vacuole-like structures are usually seen (Fig. 22). The one in the middle of the larva is the excretory cell with the excretory pore lying anterior to it and surrounded with the structures described in Fig. 21. The other two structures are probably the nerve ring and the first genital cell respectively. A small spherical area of low refractive index behind the head is sometimes seen and has been observed in a number of the earlier specimens.

A microfilaria from the pleural fluid is shown in Fig. 23. There are no significant differences between it and the microfilariae obtained from the uterus. The cells of the pleural fluid are also visible in this figure.

Microfilariae in the peripheral blood

Satisfactory photomicrographs could not be obtained of the microfilariae in the blood because of the presence of red blood cells. On visual examination, no significant differences were noted between them and the microfilariae which were found in the uterus of the female and in the pleural fluid.

Discussion

It has been possible to observe and photograph the stages in the development of the larva from a single cell. This photographic record is valuable as observations recorded by line drawings are often impressionist, and vary with the observer, cf. the observations on the structure of the heads of microfilariae as reviewed by Fulleborn (1929).

The general development of the larvae of *Litomosoides* is along the lines outlined briefly for *Loa loa* by Penel (1904) whose illustrations have been reproduced in recent publications (Chandler *et al.* 1940). Penel showed diagrammatically the development of the single cell to a microfilaria. He stated that the developing larva gradually stretched the egg-membrane to form the sheath of the microfilaria. Since then the origin of the sheaths of microfilariae and their absence in unsheathed species has been the subject of much discussion with few constructive observations. Penel's results were accepted for a time but unjust criticism of his observations have been made by modern authorities (Christenson, 1940) because he did not attempt a "critical study of the membranes", and he is misquoted as stating that the vitelline membrane forms the sheath of the microfilaria. Penel's observation was that the "membrane ovulaire" was stretched to form the sheath. The observations made by phase-contrast microscopy confirm this early observation that the entire membrane goes to form the sheath. Chitwood and his colleagues believe that there is a chitinous shell within which there is a delicate vitelline membrane, visible only "by careful study with oil immersion lenses in formalin preserved materials". In the present study there was no indication of more than one structure in the membrane, which appears to have the same structure throughout development from the two cell stage. The whole membrane certainly enters into the formation of the sheath of the mature larva. Augustine (1937) suggested that the sheath of the microfilaria was derived from the shedding of the cuticle. He found, in the adult worm of *Vagrifilaria columbigallinae*, that the external membrane was present round coiled larvae but not round extended microfilariae. Further, he found what he considered to be quantities of discarded membranes. Cross and Scott (1947) found no positive evidence that the larvae left their membranes at that stage and they observed detritus in the uterus which they presumed was the material Augustine took to be discarded membranes. More recently, Kershaw (1948) stated that the microfilariae of *Litomosoides carinii* shed their sheaths shortly after emergence from the adult worm,

and that the sheath "is always cast before the larva reaches the general spaces of the pleura". Kershaw used a rapid fixation technique on smears made from either the thoracic organs or from the adult female worm pulled across a slide. If the worm has been pulled lightly across the slide, the microfilariae are seen by phase-contrast to have sheaths, but if the adult has been subjected to greater trauma in the process, some of the microfilariae lose their sheaths. In specimens from pleural fluid all microfilariae possess sheaths when examined by phase-contrast microscopy. Similarly, all the microfilariae in the peripheral blood have sheaths although Kershaw stated that sheaths could be demonstrated only "in more than 50 per cent" of the larvae. No evidence of the casting of the membrane has been found, such as collections of empty membranes as described by Kershaw.

In the general cellular development of the larvae the outstanding feature was the formation of a body cavity with the simultaneous appearance of the subcuticular cells. The cavity gradually enlarged until it occupied the whole length of the larva and the only cellular structures which appeared to be present were the subcuticular cells. The cavity then became filled in with a number of different cells, the function and organisation of which are obscure. In the fully developed microfilariae, three round vacuole-like bodies, often containing a small granule, were usually visible. From their positions these were probably the excretory cell, the nerve ring and the genital cell. There were no gaps in the nuclear column visible by phase-contrast, as described by Kershaw in fixed and stained preparations.

Cuticular corrugations were easily observed in the specimens. When the larva was coiled within the external membrane the corrugations on the inner side were close together and on the outer side were widely spaced. Foshay (1947) employing a silver deposition technique observed corrugations on the commoner blood microfilariae, but he was less certain of them in *Litomosoides carinii*. Stefanopoulo *et al.* (1949) with phase-contrast were able to see them in *Litomosoides carinii* and stressed that the sheaths of microfilariae of *L. carinii* in the blood were easily seen.

Tne movements of the microfilariae in the pleural fluid were striking. They were able to pass rapidly through packed masses of cells in the pleural fluid from one area of the specimen to another.

It is clear from the above observations and the photomicrographs that phase-contrast microscopy is a valuable method for the study of the development of nematode larvae.

Summary

The development of the microfilariae of *Litomosoides carinii* from the ova, has been studied by phase-contrast microscopy, and recorded with photomicrographs. All stages in the development were obtained by puncturing the uteri of adult female worms at intervals along their lengths, transferring the larvae which emerged onto agar coated slides and allowing the weight of a coverglass to flatten them sufficiently for detailed examination.

The head and the tail end of the larva were differentiated at a fairly early stage and about the same time a hook-like structure appeared on the head. Subsequently a body cavity was formed within the larva which was associated with the appearance of highly refractile subcuticular cells. Later this cavity became filled with different types of cells.

Throughout its development the larva was enclosed within a membrane which was later stretched by the movement of the larva to form the sheath of the microfilaria. All the microfilariae from the adult female worms, from the pleural fluid, and from the peripheral blood of the large number of infected cotton rats examined, were seen by phase-contrast microscopy to have sheaths.

Acknowledgments

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References

Augustine, D. L., 1937.——"Observations on living "sheathed" microfilariae in the capillary circulation." *Trans. R. Soc. trop. Med. Hyg.*, **31**, 55. (W.L. 21671.)

Austin, C. R. and Smiles, J., 1948.—"Phase-contrast microscopy in the study of the fertilization of the rat egg." *J. R. micr. Soc.*, **68**, 13. (W.L. 11360.)

CHANDLER, A. C., ALICATA, J. E. and CHITWOOD, M. B., 1940, in "An introduction to nematology", Babylon, N.Y., Section II, Part II, Chapt. VI., 289.

CHRISTENSON, R. O., 1940, in "An introduction to nematology", Babylon, N.Y., Section I, Part III, Chapt. XII, 180.

CROSS, J. B. and SCOTT, J. A., 1947.—"The developmental anatomy of the fourth stage larvae and adults of *Litomosoides carinii*, a filarial worm of the cotton rat." *Trans. Amer. micr. Soc.*, **66,** 1. (W.L. 21400.)

CULBERTSON, J. T. and ROSE, H. M., 1944.—"Chemotherapy of filariasis in the cotton rat by administration of Neostam." *Science*, **99,** 245. (W.L. 19938.)

FOSHAY, L., 1947.—"The cuticular morphology of some common microfilariae." *Amer. J. trop. Med.*, **27,** 233. (W.L. 626.)

FULLEBORN, F., 1929, in "Kolle and Wassermann's Handbuch der pathogen Mikroorganismen" 3rd. Ed., 6, 1043, Jena : Fischer ; Berlin, Wien ; Urban u. Schwarzenberg.

HAWKING, F. and SEWELL, P., 1948.—"The maintenance of a filarial infection (*Litomosoides carinii*) for chemotherapeutic investigations". *Brit. J. Pharmacol.*, **3,** 285. (W.L. 3569a.)

KERSHAW, W. E., 1948.—"Observations on *Litomosoides carinii* (Travassos, 1919) Chandler 1931. 1. The development of the first-stage larva." *Ann. trop. Med. Parasit.*, **42,** 377. (W.L. 1063.)

PENEL, R., 1904.—Les filaires du sang de l'homme, Paris.

STEFANOPOULO, G. J., OVAZZA, M. and BESSIS, M., 1949.—"Utilisation du microscope à contraste de phase et de la méthode de l'ombrage en parasitologie. Application à l'étude de quelques microfilaries sanguicoles." *C. R. Soc. Biol., Paris*, **143,** 767. (W.L. 6630.)

Plate I

Fig. 1. Single cell stage. This specimen is flattened to show detail in the cytoplasm. There is an outer zone of large granules with an inner zone of fine granules round the nucleus, with short filaments between. Fig. 2. Two cell stage. The specimen is again flattened and shows large granules concentrated in the lower cell. Fig. 3. Four cell stage. The specimen is not flattened. The external membrane and "vitelline space" are clearly visible. Note the decrease in the relative amount of cytoplasm to nuclear structure. Fig. 4. Multiple cell stage—unflattened. Fig. 5. A later stage. The specimen is flattened. Nuclear structures, barely resolved, are visible. Fig. 6. This specimen shows an increase in size with the increase in the number of nuclei. Fig. 7. Commencing organisation. Large vacuole-like bodies containing a small dark structure are visible and also two or three large dark granules. Fig. 8. The outline of the larva is irregular and there is an indentation on the right side, which is the first stage in the shaping of the larva. Fig. 9. This shows a further increase in size and the curvature of the larva becoming more marked. A broad end and a narrow end can be distinguished, the former going to form the head and the latter the tail. (All photomicrographs are X 1300 except Fig. 23 which is X 800, and were taken with the standard C. T. & S. phase-contrast equipment.)

Plate II

Fig. 10. The beginning of a hook structure is seen on the head of the larva. Figs. 11 to 15. These figures show the formation of a body cavity, commencing on the dorsum near the tail and gradually extending the entire length of the larva. Highly refractile subcuticular cells, seen first in Fig. 11 form almost the entire cellular structure of the larva in Fig. 15. Further development of a hook on the head is shown in Figs. 13 and 14. The first stage at which movement of the larva was detected is that shown in Fig. 11. Figs. 16 to 18. These stages are all actively moving, the larvae wriggling about within the external membranes. The body cavity is becoming filled in with several types of cells.

Plate III

Figs. 19 to 21. The external membrane is gradually being stretched by the movements of the larva. The hook on the head is seen in Figs. 19 and 20. The excretory pore is visible in Fig. 21, surrounded by a dark area and then a light area. Fig. 22. The larva has become fully extended within the external membrane. Three clear areas are seen in the body, the anterior being probably the nerve ring, the one in the middle the excretory cell and the posterior the genital cell. Just behind the head a small clear spot is noted which is visible in a percentage of the microfilariae. Fig. 23. A microfilaria from the pleural fluid. The clear area behind the head noted in Fig. 22 is much larger in this specimen. Cells from the pleural fluid are visible.

Plate I

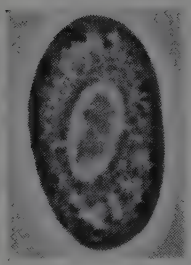


Fig. 1

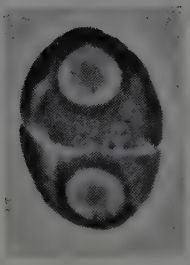


Fig. 2

Fig. 3

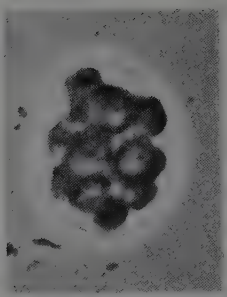


Fig. 4

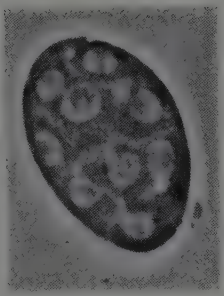


Fig. 5

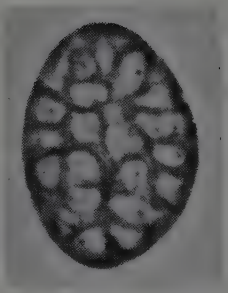


Fig. 6

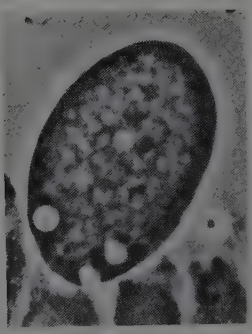


Fig. 7

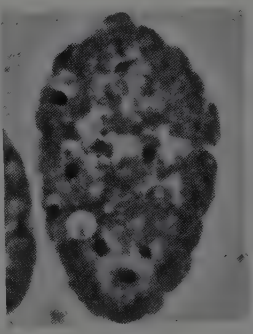


Fig. 8

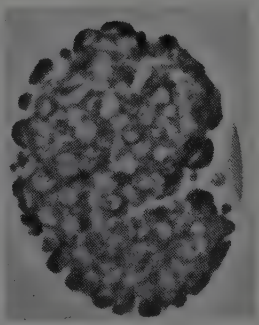


Fig. 9

Plate II

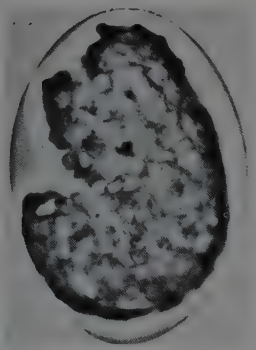


Fig. 10

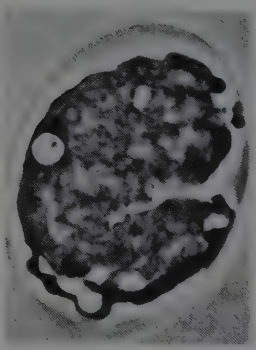


Fig. 11

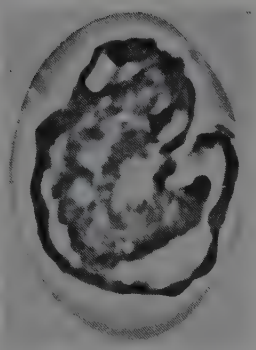


Fig. 12

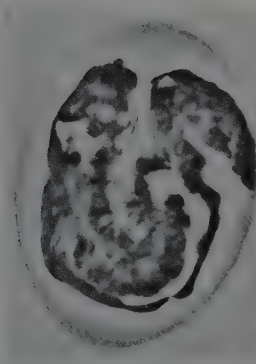


Fig. 13

Fig. 14

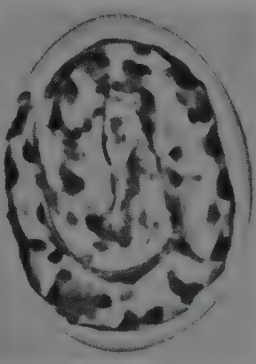


Fig. 15

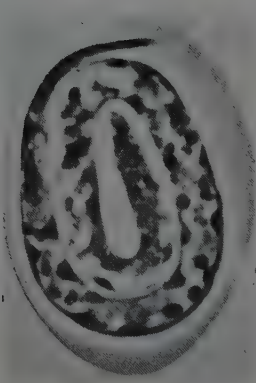


Fig. 16

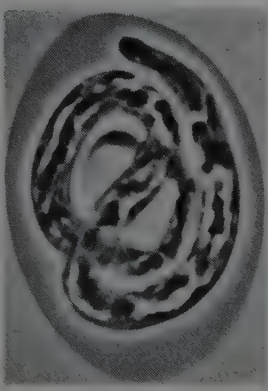


Fig. 17

Fig. 18

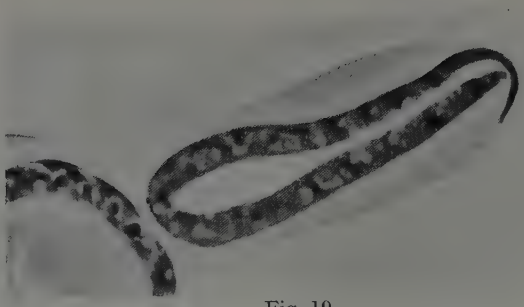


Fig. 19

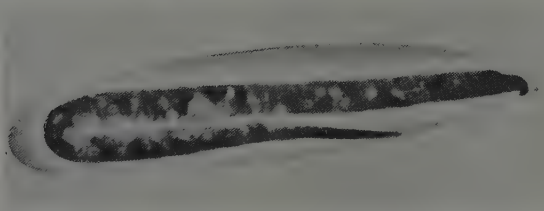


Fig. 20

Fig. 21

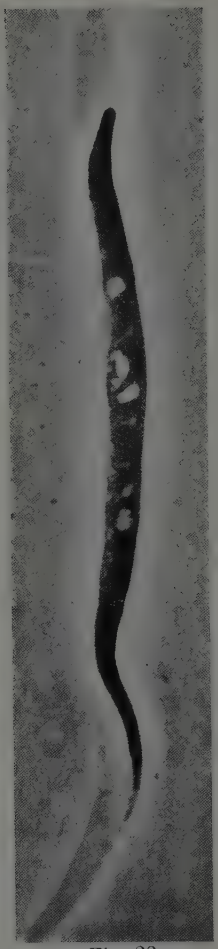


Fig. 22

Fig. 23

Journal of Helminthology, Vol. XXX, No. 1, 1956, *pp.* 33–40.

The Effect of Light on the Emergence of *Cercaria pygocytophora*, a Furcocercaria from *Planorbis carinatus*

By Helga M. T. Frankland, Ph.D.
University College of North Staffordshire

It is well known that many cercariae have a periodic rhythm of emergence from the snail host. It must be assumed that the peak period of emergence is, for each species, the most favourable for further development and accordingly may give some clue as to tth life history.

The causes of this periodicity are varied and in many cases have not been established. In some species there is evidently an internal rhythm either dependent on developmental rhythms of the trematode (Cort, 1922), or perhaps on the activation of the host snail as suggested by Kendall and McCullough (1951).

In many species, however, external factors determine the rhythm, at least in part. The most important of these are heat and light, both tending to produce in nature a diurnal rhythm. From the work of Giovanolla (1936a), Kuntz (1947), and Schreiber and Schubert (1949) it is clear that heat and light each play a part in stimulating emergence of the cercariae of *Schistosoma mansoni*. Brackett (1940) came to a similar conclusion working with *Cercaria elvae*. Rees (1931) was able to show that light alone was the stimulating factor in the case of the daytime-emerging *Cercaria limbifera* since emission was inhibited by keeping the hosts in continuous darkness. Other species have been shown to be similarly dependent on light for their emergence rhythm by reversing the normal light and dark periods. Thus Giovanolla (1936b) was able to reverse the time of emergence of the cercaria of *Diplostomum flexicaudum* and an unidentified furcocercaria, both of which continued to emerge in the light when the period of illumination was reversed. Rees (1948) got the same result with *Cercaria purpurae*. Conversely Giovanolla (1936b) and Olivier (1951) found that a cercaria of the sub-family Reniferinae and the cercaria of *Schistosomatium douthitti* respectively continued to emerge in the dark when the light period was reversed.

Dubois (1929) considered that stimulation of emergence by light or dark was not necessarily associated with the corresponding phototactic reaction and Krull (1931) supported this view by his finding that the dark-emerging cercariae of *Pneumonoeces medioplexus* showed no noticeable phototaxis. However Cort (1922) showed a day-emerging echinostome cercaria to be positively phototactic, Brackett (1940) found various schistosome cercariae to emerge during the day and to be positively phototactic, and Rees (1948) found *Cercaria purpurae* to behave in the same way.

The present experiment was undertaken to determine whether the emergence of *Cercaria pygocytophora* was influenced by light and whether it was photosensitive. I am indebted to Dr. T. T. Macan for assistance with the identification of the snail hosts.

The Cercaria

Brown's (1931) *C. pygocytophora* is closely similar in size and form to two other species, *C. burti* of Miller (1923, 1926) and *C. helvetica* XXXI of Dubois (1929), the principal difference being in the excretory tubules: *C. burti* has a transverse, commissural canal posterior to the ventral sucker whereas *C. helvetica* XXXI has in addition a transverse commissure anterior to the ventral sucker. Wesenberg-Lund (1934) however described specimens assigned by him to *C. helvetica* XXXI in which he could only see the anterior commissure. He thought that probably both species had in reality two commissures. *C. pygocytophora* has no transverse commissures, but, on Wesenberg-Lund's interpretation, these may merely be difficult to detect and if they are present, then all three species may be identical.

Table I

Measurements of living C. pygocytophora

Organ	Brown (1931)	Present Work
Body length	130μ	144μ
Body width	60μ	65μ
Tail stem length	140μ	144μ
Tail stem width	—	50μ
Tail furca length	150μ	151μ

The cercaria used in the present work agrees in its morphology almost completely with Brown's (1931) *C. pygocytophora*. The measurements of living material correspond, as will be seen from Table 1, when allowance is made for compression by the coverslip.

The only disagreements between the present cercaria and Brown's are, firstly, that in the former unpigmented eye-spots were seen antero-laterally to the ventral sucker, these not being mentioned by Brown (1931). However this is not regarded as important because although Miller (1923, 1926) described *C. burti* as being without eye-spots Cort and Brooks (1928) spoke of the same cercaria as having unpigmented eye-spots. Secondly, Brown only described four hair-like projections down each side of the tail stem and none on the body, whereas the present cercaria had approximately ten down each side of the tail stem and three on each side of the body. These are difficult to see and again the discrepancy is probably unreal. Miller (1923, 1926) said nothing of hair-like projections on *C. burti* while Cort and Brooks (1928) recorded six along each side of the tail stem.

The present cercaria has the same host, *Planorbis carinatus*, as *C. pygocytophora* and like this latter, as will be shown later, is negatively phototactic. Accordingly this cercaria is regarded as *C. pygocytophora* and it is thought possible that *C. burti*, *C. helvetica* XXXI, and *C. pygocytophora* may all belong to one species.

Material and Methods

The snails used for the experiments were naturally infected *Planorbis carinatus* collected from a lake near Keele, N. Staffordshire.

Experiment 1

Ten snails found to be emitting cercariae satisfactorily were selected for the experiment which was carried out in early July. The snails were kept individually in 2 in. $\times \frac{3}{4}$ in. tubes, almost full of water and corked. These were partially immersed in a water bath whose temperature was usually 22°C., the maximum variation being from 20°C. to 24°C. The apparatus was set up near a north window and an overhead light burnt from 8 p.m. to 8 a.m. From 8 p.m. to 8 a.m. Snails I—V were in the dark (in tubes painted black) and Snails VI—X were exposed to light (in unpainted tubes). From 8 a.m. to 8 p.m. Snails I—V were exposed to light and Snails VI—X were in blackened tubes.

When the snails were transferred to new tubes the cercariae which had emerged from each snail during the preceding period were

poured off under standard conditions and fixed for subsequent counting. A direct count of each batch of cercariae was made under a hand lens in a petri dish standing on black, squared paper. Counts were taken of twelve hours output at the beginning of the experiment and at shorter intervals later on.

Cercariae from each snail were examined to make sure that all belonged to the same species. No food was given to the snails during the experiment, but all were healthy at its conclusion.

Experiment 2

To test photosensitivity a number of vigorous cercariae were poured into a Petri dish standing half on black and half on white paper ; half the lid was also blackened so that there was a dark and a light zone in the dish. A lamp was placed directly overhead, the temperature of an adjacent dish being 20°C., and the whole left for two hours.

At the end of this time a piece of card was quickly placed on edge in the dish along the frontier between dark and light zones. Fixative was then poured into each side, the dish placed on the black, squared, counting paper, and the number of cercariae in each zone counted.

Results

Experiment 1

The results of *Experiment* 1 are set out in Table 2 in which the actual numbers of cercariae emerging from each snail are shown.

Table 3 gives the results expressed as a percentage of total daily output.

From these tables it is clear that the vast majority of the cercariae emerged under conditions of darkness and that, in general, the greatest emergence occurred between the second and fourth hours after the onset of dark conditions. So far as could be ascertained the behaviour of the snails was not modified by a change of illumination : on opening the tube, they were usually to be found attached near the top regardless of whether light was available or not.

Experiment 2

The number of cercariae found respectively in the dark and light zone of the Petri dish after two hours is shown in Table 4.

TABLE II

Numbers of cercariae emerging during Exp. 1

Date	Period	Temp. °C.	No. and Diam. of Snails started in Dark					Light		No. and Diam. of Snails started in Light				
			I 10 mm.	II 10 mm.	III 10 mm.	IV 6 mm.	V 10 mm.	I—V	VI—X	VI 12 mm.	VII 13 mm.	VIII 13 mm.	IX 10 mm.	X 10 mm.
July 6—7	8 p.m. — 8 a.m.	22	1,478	2,238	902	784	992	—	+	457	1,552	791	1,069	1,030
	8 a.m. — 8 p.m.	22	320	150	438	291	517	+	—	2,587	1,752	1,954	1,704	1,379
	24 hours total		1,798	2,388	1,340	1,075	1,059			3,044	3,304	2,745	2,773	2,409
July 7—8	8 p.m. — 8 a.m.	22	1,734	1,994	773	1,033	772	—	+	171	1,226	659	82	68
	8 a.m. — 8 p.m.	22	303	18	284	397	674	+	—	2,085	2,052	2,476	1,420	1,587
	24 hours total		2,037	2,012	1,057	1,430	1,446			2,256	3,278	3,135	1,502	1,655
July 8—9	8 p.m. — 8 a.m.	22	1,449	1,448	575	870	825	—	+	45	1,509	228	239	45
	8 a.m. — 8 p.m.	21—24	171	135	90	331	426	+	—	1,739	2,950	2,258	2,236	1,366
	24 hours total		1,620	1,583	665	1,201	1,251			1,784	4,459	2,486	2,475	1,411
July 9—10	8 p.m. — 8 a.m.	23	1,345	1,680	628	1,049	686	—	+	52	1,559	145	112	49
	8 a.m. — 8 p.m.	22	174	14	158	207	349	+	—	1,581	2,950	1,517	1,661	1,003
	24 hours total		1,519	1,694	786	1,256	1,035			1,633	4,509	1,662	1,773	1,052
July 10—11	8 p.m. — 8 a.m.	22	1,203	1,768	590	1,056	421	—	+	144	1,778	164	70	27
	8 a.m. — noon	22	38	10	0	46	23	+	—	881	1,095	967	825	238
	noon — 4 p.m.	21.5	3	0	5	15	62	+	—	410	574	259	121	457
	4 p.m. — 8 p.m.	21	185	75	151	221	169	+	—	128	492	141	130	207
	8 a.m. — 8 p.m.	21—22	226	85	156	282	254	+	—	1,419	2,161	1,367	1,076	902
	24 hours total		1,429	1,853	746	1,338	675			1,563	3,939	1,531	1,146	929
July 11—12	8 p.m. — 8 a.m.	22	904	1,227	499	584	374	—	+	48	1,106	104	52	36
	8 a.m. — 9 a.m.	21	3	19	5	17	6	+	—	1	59	0	12	3
	9 a.m. — noon	21	18	10	9	4	17	+	—	635	636	154	699	407
	noon — 4 p.m.	21	46	1	14	2	26	+	—	383	418	316	180	121
	4 p.m. — 8 p.m.	20	183	64	156	140	45	+	—	112	323	40	55	70
	8 a.m. — 8 p.m.	20—21	250	94	184	163	94	+	—	1,131	1,436	510	946	601
	24 hours total		1,154	1,321	683	747	468			1,179	2,542	614	998	637

TABLE III

% daily output of cercariae in each period during Exp. 1

Date	Period	Snails started in Dark					Snails started in Light				
		I	II	III	IV	V	VI	VII	VIII	IX	X
July 6—7	8 p.m. — 8 a.m.	82.2	93.7	67.3	72.9	93.7	15.0	46.9	28.8	38.5	42.7
	8 a.m. — 8 p.m.	17.8	6.3	32.7	27.1	6.3	85.0	53.1	71.2	61.5	57.3
July 7—8	8 p.m. — 8 a.m.	85.1	99.1	73.1	72.2	53.4	7.6	37.4	21.0	5.5	4.1
	8 a.m. — 8 p.m.	14.9	0.9	26.9	27.8	46.4	92.4	62.6	79.0	94.5	95.9
July 8—9	8 p.m. — 8 a.m.	89.4	91.5	86.4	72.4	65.9	2.5	33.8	9.2	9.7	3.2
	8 a.m. — 8 p.m.	10.6	8.5	13.6	27.6	34.1	97.5	66.2	90.8	90.3	96.8
July 9—10	8 p.m. — 8 a.m.	88.5	99.2	79.9	83.5	66.3	3.1	34.6	8.7	6.3	4.7
	8 a.m. — 8 p.m.	11.5	0.8	20.1	16.5	33.7	96.9	65.4	91.3	93.7	95.3
July 10—11	8 p.m. — 8 a.m.	84.2	95.5	79.1	78.9	62.4	9.2	45.1	10.7	6.1	3.0
	8 a.m. — noon	2.7	0.5	0.0	3.4	3.4	56.4	27.8	63.2	72.0	25.2
	noon — 4 p.m.	0.2	0.0	0.7	1.1	9.2	26.2	14.6	16.9	10.6	49.2
	4 p.m. — 8 p.m.	12.9	4.0	20.2	16.6	25.0	8.2	12.5	9.2	11.3	22.6
	8 a.m. — 8 p.m.	15.8	4.5	20.9	21.1	37.6	90.8	54.9	89.3	93.9	97.0
July 11—12	8 p.m. — 8 a.m.	78.3	92.9	73.1	78.1	80.0	4.0	43.6	16.9	5.2	5.6
	8 a.m. — 9 a.m.	0.3	1.4	0.7	2.3	1.3	0.1	2.3	0.0	1.2	0.5
	9 a.m. — noon	1.5	0.8	1.3	0.5	3.6	53.9	25.0	25.1	70.1	63.9
	noon — 4 p.m.	4.0	0.1	2.0	0.3	5.5	32.5	16.4	51.5	18.0	19.0
	4 p.m. — 8 p.m.	15.9	4.8	22.9	18.8	9.6	9.5	12.7	6.5	5.5	11.0
	8 a.m. — 8 p.m.	21.7	7.1	26.9	21.9	20.0	96.0	56.4	83.1	94.8	94.4

It will be seen that the cercaria is negatively phototactic.

TABLE IV

Phototaxis of C. pygocytophora

Condition of Zone	No. of Cercariae
Dark	631
Light	17

DISCUSSION

The results of *Experiment* I show clearly that the onset of darkness stimulates the emergence of *C. pygocytophora* from *P. carinatus* regardless of the time at which light is cut off. Though temperature variations may also play a part in nature, a rhythm of emergence dependent on periodic darkness occurs when the temperature is constant.

Although emergence was heaviest between two and four hours after the onset of darkness, it continued throughout the dark period. It is, however, noteworthy that some cercariae also emerged from all the experimental snails during the light period, most emerging during the last four of the twelve hours of light. It is possible that mature cercariae become pent up in the snail during the inhibitory period. This may account for the less well marked rhythm of Snail VII which was, judged by the number of cercariae emerging, the most heavily infected of the ten snails. That this cannot be the whole explanation is seen from a study of the yield of Snail V. This snail, though not particularly heavily infected, showed a weakening of the rhythm during the mid-part of the experiment. It is probable that, as Kendall and McCullough (1951) suggest, the condition of the snail itself plays a part.

The negative phototaxis of *C. pygocytophora*, already referred to by Brown (1931), is very pronounced and suggests that the second intermediate host is either a nocturnal or bottom dwelling species.

SUMMARY

1. *C. pygocytophora* emerges from *P. carinatus* predominantly in the dark, independently of the time of day, and in the absence of temperature variation.

2. *C. pygocytophora* shows strong negative phototaxis.

References

Brackett, S., 1940.—"Studies on schistosome dermatitis : VI. Notes on the behaviour of schistosome cercariae." *Amer. J. Hyg.* **31,** Sec. D., 64–73.

Brown, F. J., 1931.—"Some freshwater larval trematodes from Cheshire." *Parasitology,* **23,** 88–98.

Cort, W. W., 1922.—"A study of the escape of cercariae from their snail hosts." *J. Parasit.* **8,** 177–184.

Cort, W. W., and Brooks, S. T., 1928.—"Studies on the holostome cercariae from Douglas Lake, Michigan." *Trans. Amer. Micr. Soc.,* **47,** 179–221.

Dubois, G., 1929.—"Les cercaires de la région de Neuchâtel. *Bull. Soc. Neuchâtel. Sci. Nat.,*" **53,** Année 1928, 1–178.

Giovanolla, A., 1936a.—"Some observations on the emission of cercariae of *Schistosoma mansoni* (Trematoda : Schistosomatidae) from *Australorbis glabratus.*" *Proc. Helm. Soc. Wash.,* **3,** 60–61.

———1936b.—"Inversion in the periodicity of emission of cercariae from their snail hosts by reversal of light and darkness." *J. Parasit.,* **22,** 292–295.

Kendall, S. B., and McCullough, F. S., 1951.—"The emergence of the cercariae of *Fasciola hepatica* from the land snail, *Limnaea truncatula.*" *J. Helminth.,* **25,** 77–92.

Krull, W. H., 1931.—"Life history studies on two frog lung flukes, *Pneumonoeces medioplexus* and *Pneumobites parviplexus.*" *Trans. Amer. Micr. Soc.,* **50,** 215–277.

Kuntz, R. E., 1947.—"Effect of light and temperature on emergence of *Schistosoma mansoni* cercariae." *Trans. Amer. Micr. Soc.,* **66,** 37–49.

Miller, H. M., 1923.—"Notes on some furcocercous larval trematodes." *J. Parasit.,* **10,** 35–46.

———1926.—"Comparative studies on furcocercous cercariae." *Illinois Biol. Monogr.,* **10,** No. 3, 1–112.

Olivier, L. J., 1951.—"The influence of light on the emergence of *Schistosomatium douthitti* cercariae from their snail host." *J. Parasit.,* **37,** 201–204.

Rees, G., 1931.—" Some observations and experiments on the biology of larval trematodes." *Parasitology,* **23,** 428–440.

———1948.—"A study of the effect of light, temperature and salinity on the emergence of *Cercaria purpurae* Lebour from *Nucella lapillus* (L). *Parasitology,* **38,** 228–242.

Schreiber, F. G., and Schubert, M., 1949.—"Experimental infection of the snail *Australorbis glabratus* with the trematode *Schistosoma mansoni* and the production of cercariae." *J. Parasit.,* **35,** 91–100.

Wesenberg-Lund, C., 1934.—"Contributions to the development of the Trematoda Digenea : II. The biology of the freshwater cercariae in Danish freshwaters." *Mem. Acad. Roy. Sci. Lett. Danemark.* Sect. Sci. 9th Ser. **5,** No. 3.

Journal of Helminthology, Vol. XXX, No. 1, 1956, *pp.* 41–50.

* On a New Acanthocephalan, *Echinopardalis lerouxi* n.sp., from a Jackal (*Canis adustus*) in Central Africa

By Bisseru, M.Sc., Ph.D.

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

The acanthocephalans previously reported from the Canidae are *Echinopardalis atrata* Meyer, 1931, *Macracanthorhynchus hirudinaceus* (Pallas, 1781), *M. catulinus* Kostylew, 1927, *Moniliformis moniliformis* (Bremser, 1811), *Oncicola canis* (Kaupp, 1909), *Pachysentis canicola* Meyer, 1931, *P. ehrenbergi* Meyer, 1931, and *P. procumbens* Meyer, 1931. Meyer in his monograph on the Acanthocephala (1932–33 : 251) reports in an appendix to the family Pachysentidae (synonymous with Oligacanthorhynchidae—Van Cleave (1953)), *Echinorhynchus* (s.l.) *pachyacanthus* Sonsino, 1899 (=*Prosthenorchis* (s.l.) *pachyacanthus* (Sonsino) in Travassos, 1917), a parasite of *Canis aureus* in Egypt. Witenberg (1934) cites *Prosthenorchis pachyacanthus* as an occasional parasite of dogs and cats in Palestine, but maintains that the normal host is a Jackal. Neveu-Lemaire (1936) lists this parasite as *Echinopardalis pachyacanthus* (Sonsino, 1899), (syn *Prosthenorchis pachyacanthus* (Sonsino, 1899)).

Through the courtesy of Dr. P. L. LeRoux the writer has had the privilege of examining the Acanthocephala which he collected in Northern Rhodesia in June, 1934. The material was obtained from the intestine of two Jackals, *Canis adustus* (syn. *Thos adustus*), and is represented by numerous mature individuals of both sexes, so that a complete study of the morphological characters of the species has been possible. The material which was preserved in glycerine-alcohol and probably fixed in formalin, was in good histological condition. It was studied in whole mounts, dissections and serial sections. The specimens were treated with trisodium phosphate (Van Cleave and Ross, 1947), stained with alcoholic borax-carmine and acetic alum-carmine and prepared as permanent microscopical mounts in balsam. Studies have also been made of specimens cleared in glycerine and mounted in glycerine-jelly after treatment

* Part of a Thesis approved by the University of London for the award of the Ph.D. degree.

with trisodium phosphate. The sections were stained with Heidenhain's iron haematoxylin or with Ehrlich's acid haematoxylin counterstained with eosin.

In a cursory examination of the material the probocis seemed to conform in general appearance to that of either the genus *Oncicola* Travassos, 1917 or *Hamanniella* Travassos, 1915, but a more detailed study of these worms has revealed profound morphological differences from these genera, and a close relationship with the species of the genus *Echinopardalis* Travassos, 1918 has been observed. Hence the worms are recognised as belonging to a new species of *Echinopardalis*, which is here described as *Echinopardalis lerouxi* n.sp., and this represents the third record of the occurrence of this Acanthocephalan genus from the Old World.

OLIGACANTHORHYNCHIDAE Southwell and Macfie, 1925
emended Meyer, 1931.

Echinopardalis Travassos, 1918.

Echinopardalis lerouxi n.sp.

This species is characterised by a marked sexual dimorphism—the males are shorter than the females, and have their posterior extremity more or less rounded or abruptly truncated, while in the female the body tapers to a blunt-pointed appendix or papilla-like projection (Genital papilla Van Cleave, 1945), which begins posterior to the female genital opening. In some female individuals cement material had hardened around the genital extremity forming a "copulatory cap". This cap was easily removed with the aid of a needle before staining the worms. Both males and females have an elongate robust body, with the anterior half slightly inflated. They are rounded in cross-section, relatively smooth, except for very slight transverse wrinking of the trunk towards the praesoma, which is especially noticeable in some contracted specimens. In normally extended specimens of both sexes a very slight ventral flexure of the anterior end of the body is noticeable. In contracted specimens the neck and proboscis may sometimes be totally retracted into the front part of the trunk, while in partly retracted and fully extended individuals the base of the neck and the anterior end of the trunk are introverted within the front end of the body.

The cuticular body wall is very thin and the musculature relatively weak. The lacunar system of the body is comparatively poorly developed,—a dorsal and a ventral vessel are present, but the circular vessels are uneven in their distribution. The subcuticular amoeboid

giant nuclei appear commonly in the anterior half of the body, surrounding the main longitudinal vessels.

The females are 22 to 47 mm. long with a diameter of 1.5 to 2.2. mm. in the anterior half of the body. The corresponding measurements in the male are 16 to 28 mm. long and 1.2 to 1.9 mm. wide. The proboscis is globular or very slightly elongated, 0.43 to 0.66 mm. in diameter. The neck is short. The proboscis armature consists of six spiral rows with six relatively strong hooks in each row, each hook being provided with one or more processes. In the anterior two hooks of each row the root process (Fig. 3) has a long anteriorly directed extension in addition to a very short posterior process. The root of the 3rd hook is always asymmetrically bent towards the left with the tip sometimes bent inwards and posteriorly, whereas that of the 4th hook is bent towards the right with the root process of the 3rd hook overlapping it. The roots of the 5th and 6th hooks are broad, of irregular shape and without any special antero-posterior continuations. The first two hooks in each row are distinctly stouter than the rest. While a few of the hooks are very much recurved, some approaching a semi-circle, a very large number of them have a characteristic fish-hook like tip. The length of the hook is taken from the tip of the hook to the point where the hook and root meet. The basal diameter of the hook is taken at the point where the blade and root meet. The dimensions of the hooks are given in Table I.

TABLE I

Number of hook	Length of hooks	Basal diameter of hooks	Over-all length of roots
1	0·13 —0·16	0·043—0·058	0·126—0·18
2	0·128—0·18	0·039—0·060	0·160—0·20
3	0·110—0·136	0·033—0·039	0·122—0·147
4	0·108—0·129	0·028—0·032	
5	0·081—0·093	0·025—0·028	
6	0·068—0·088	0·021—0·025	

All measurements in millimeters.

The muscular elongate proboscis receptacle, whose posterior extremity has a blunt rounded termination, is a sac-shaped structure measuring 1.05 to 1.35 mm. long, inserted near the base of the 6th hooks. It is very thick dorsally and very thin ventrally. The two invertor muscles which are attached to the tip of the proboscis extend backwards side by side within the proboscis receptacle.

Fibres from the invertors pass through the wall of the receptacle and continue for about 1.9 to 2.4 mm. through the body cavity as a single dorsal and two ventral retractors of the proboscis receptacle. In addition there are a number of circularly arranged dorsal, ventral and lateral receptacle protrusers, which arise from the neck wall, and insert at the posterior end of the wall of the receptacle. The longitudinally-directed neck retractors arising near the posterior boundary of the neck encircle the other muscles of the praesoma and surround the two lemnisci as the compressors of the lemnisci.

The brain, which in this species is an ovoid mass 0.175 to 0.190 mm. long, is situated more or less in the middle of the length of the proboscis receptacle, about 1.1 mm. from the anterior extremity of the body. The whole cellular mass is enclosed within the proboscis receptacle, in close contact with its ventral wall. The individual ganglionic cells are ovoid in shape with a breadth of 0.025 to 0.033 mm. The conspicuous nuclei in these cells have a diameter of 0.01 to 0.012 mm.

The lemnisci are very long, round in cross-section, with a central canal and without any narrowing in the posterior part. They measure 14 to 18 mm. long and 0.16 to 0.24 mm. wide, but this width increases to about 0.3 mm. in areas where nuclei occur. The lemnisci are usually much looped. They are provided with 5 to 6 nuclei measuring up to 0.4 mm. long and distributed in the anterior and middle regions of the lemnisci. In male worms the lemnisci sometimes extend to the end of the first testis.

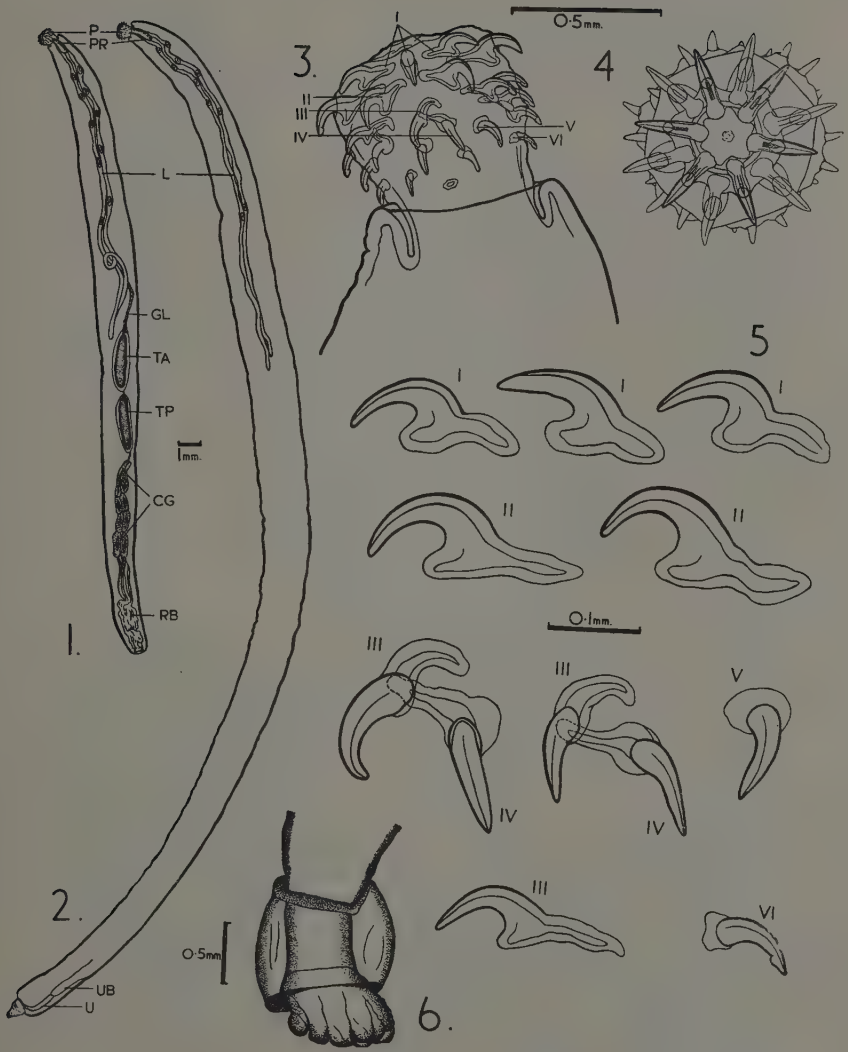
In both sexes the excretory or protonephridial organs are fairly similar in structure and closely applied to the reproductive system. Each organ or bladder is relatively large and the protonephridial system consists of a branching mass of bulbous structures. In females the protonephridial organs lie near the opening of the

Echinopardalis lerouxi n.sp.

Fig. 1.—Male, showing lemnisci and genital organs. Fig. 2.—Female, showing lemnisci and genital organs. Fig. 3.—Proboscis and anterior end of trunk. Fig. 4.—End-on view of proboscis, showing terminal hooks in each spiral row. Fig. 5.—Individual hooks and their roots, seen under higher magnification. Fig. 6.—Extruded bursa of male.

Abbreviations used in Figs. 1–6.

CG = Cement glands; **GL** = Genital ligament; **P** = Proboscis; **PR** = Proboscis receptacle; **L** = Lemnisci; **RB** = Retracted bursa; **TA** = Testis anterior; **TP** = Testis posterior; **U** = Uterus; **UB** = Uterine bell.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uterine bell and the excretory duct opens into the uterus. In the male the protonephridial organs and excretory bladder are found at the end of the hinder pair of cement glands, with the organs lying on the anterior side of the bladder. The excretory duct here proceeds dorsally to the two vasa efferentia, and opens into the urinogenital canal.

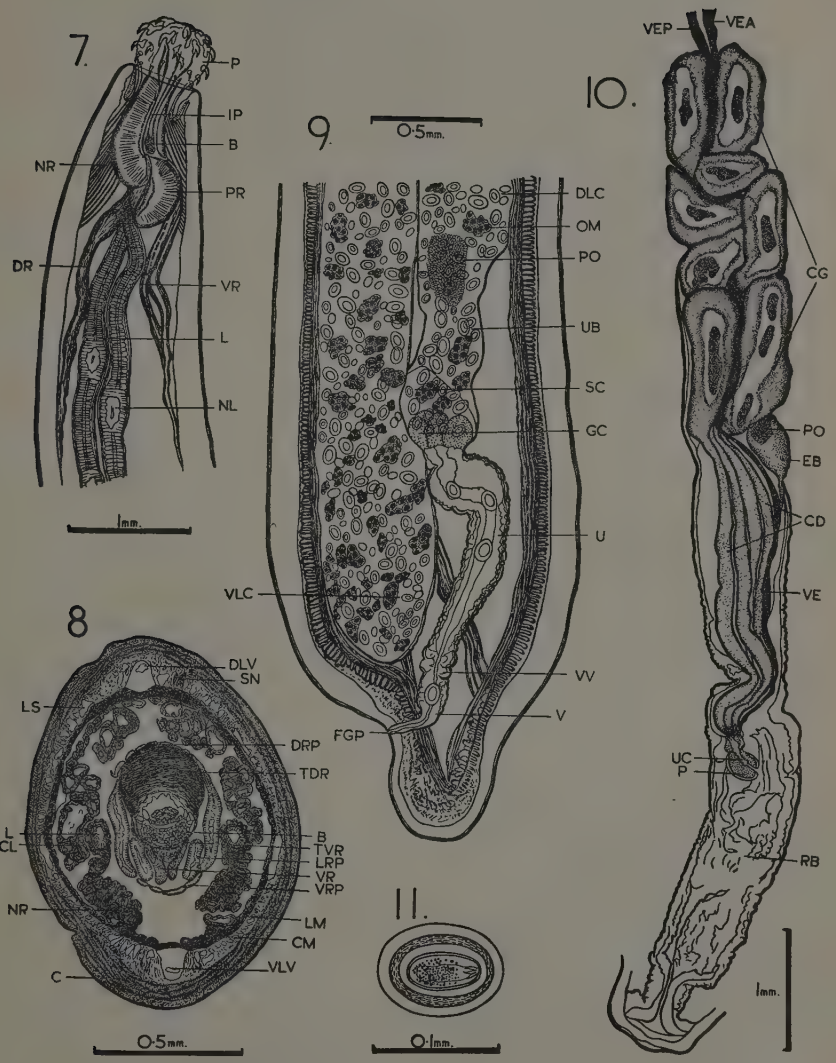
The male genitalia occupy the posterior half of the body, the anterior testis beginning at about the middle of the body-length. The testes which are attached by a genital ligament to the base of the proboscis receptacle, are enclosed in a broad sheath. They are elongate structures, lying one behind the other, with an appreciable distance separating them. Both testes are of about equal size, 1.6 to 2.4 mm. long by 0.37 to 0.48 mm. broad, excluding the testicular sheath. A row of 4 pairs of ovoid cement glands follow the testes, extending as a series for a distance of 2.9 to 4 mm. These glands each containing a single large nucleus, are variable in shape and size, and the row is sometimes bent. Each gland has a separate efferent cement duct which enters the copulatory apparatus within the bursa. The penis is a solid mass of muscular tissue, surrounding the terminal portion of the urinogenital canal, which is formed by the union of the vas deferens, eight cement ducts and excretory duct. The copulatory bursa measures 1.32 mm. long and when extruded represents a closed hand.

Echinopardalis lerouxi n.sp.

Fig. 7.—Praesoma. Fig. 8.—Section through the proboscis receptacle, showing brain, muscles, etc. Fig. 9.—Posterior end of female showing genital tract, ligament sacs and protonephridial organ. Fig. 10.—Posterior end of male showing genitalia, (testes not shown) protonephridial organ and excretory bladder. Fig. 11.—Embryo.

Abbreviations used in Figs. 7–11.

B = Brain; **C** = Cuticle; **CD** = Cement ducts; **GC** = Cement glands; **CL** = Compressors of lemnisci; **CM** = Circular muscle fibres; **DLV** = Dorsal lacunar vessel; **DLC** = Dorsal ligament sac; **DR** = Dorsal retractor muscle; **DRP** = Dorsal receptacle protrusers; **EB** = Excretory bladder; **GC** = Guard cells; **FGP** = Female genital pore; **IP** = Invertor muscles of proboscis; **L** = Lemnisci; **LM** = Longitudinal muscle fibres; **LS** = Lacunar sinus; **LRP** = Lateral receptacle protrusers; **NR** = Neck retractors; **NL** = Nuclei of lemnisci; **OM** = Ovarian masses; **P** = Proboscis; **PI** = Penis; **PO** = Protonephridial organ; **PR** = Proboscis receptacle; **RB** = Retracted bursa; **SC** = Selecting chamber; **SN** = Skin nuclei; **TDR** = Thick dorsal wall of receptacle; **TVR** = Thin ventral wall of receptacle; **U** = Uterus; **UB** = Uterine bell; **UC** = Urinogenital canal; **V** = Vagina; **VV** = Vaginal valves; **VR** = Ventral retractor muscle; **VRP** = Ventral receptacle protrusers; **VLV** = Ventral lacunar vessel; **VLC** = Ventral ligament sac; **VE** = Vas efferens; **VEA** = Vas efferens of anterior testis; **VEP** = Vas efferens of posterior testis.


7.
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With most female individuals the genital tract is obscured by the accumulation of embryos in the posterior body region. The uterine bell of the female apparatus shows an attachment to the ligament sacs. The posterior narrower part of the uterine bell leads into a selecting chamber (see Witenberg, 1938), which has a complex of guard or selecting cells. The oviduct is a thick-walled muscular tube opening into the prominent muscular uterus, which leads to the short vagina. The structures of the female genitalia are contractile and their shape has been found to vary. The female genital aperture is sub-terminal. The embryonated eggs taken from the body of the female and examined in distilled water measure, including membranes, 0.095 to 0.108 mm. long by 0.07 to 0.082 mm. wide. The outer membrane of the egg is smooth and transparent, the inner one is thick, while that covering the embryo is much thinner.

Relationships

On the basis of the following characters the present specimens are referred to the genus *Echinopardalis* Travassos, 1918 :—

Body relatively long, with mature individuals usually having a dorsal appendix posterior to the female genital opening ; lacunar system comparatively weakly developed, amoeboid giant nuclei lying in the vicinity of the main longitudinal vessels, proboscis armed with six spiral rows, each of six hooks, most of which have a large asymmetrical root extending both anteriorly and posteriorly from the base of the hook ; lemnisci long, cylindrical, reaching posteriorly to the testes ; male reproductive organs occupying about one half of body length ; testes elongate ; cement glands eight in number, more or less paired in a row immediately posterior to testes ; protonephridial organs are present ; embryos broadly ellipsoidal, with three membranes of which the outer one is not compacted.

The records of the members of the genus *Echinopardalis* have so far been restricted to South America, Egypt and India. Van Cleave (1953) in his Monograph on the Acanthocephala of North American Mammals reports *Echinopardalis macrurae* Meyer, 1931 from *Lynx rufus*, but according to him "the host was residing in a zoo, the source of infection is wholly unkown and the parasite cannot be attributed with certainty to the endemic fauna of North America".

E. macrurae Meyer, 1931, *E. pardalis* (Westrumb, 1821), Travassos, 1917, and *E. decrescens* Meyer, 1931 are three species previously reported from Felidae in South America. The species *E. atrata* Meyer, 1931 was obtained from *Herpestes ichneumon*, *Felis catus* and *Canis vulpecula* in Egypt. *Echinopardalis bangalorensis* Pujatti, 1951 is an immature acanthocephalan found encysted in the neck of a pheasant, *Francolinus pondicerianus*, at Bangalore in South India. The normal host here is thought to be probably a carnivore.

Echinopardalis lerouxi is clearly distinct from the three South American forms all of which have a smaller body size, larger hooks on the proboscis and very much smaller embryos. Amongst other differences *E. bangalorensis* has very much larger hooks and a small proboscis. The writer was for sometime inclined to believe that *E. lerouxi* was similar to *E. atrata* owing largely to the fact that the latter species, as described by Meyer (1931), was not fully mature, but a detailed study and comparison have revealed a number of features in which the Central African material differs distinctly from the Egyptian species. The hooks as given by Meyer (1931), are very much larger, and the proboscis is comparatively smaller than in the present specimens. The disposition of the 3rd and 4th hooks and their roots show marked differences. Moreover, the proboscis in *E. atrata* has a different form, and there is no introversion of the neck in the anterior part of the trunk. Finally other differences lie in the fact that *E. atrata* lacks an appendix posterior to the female genital opening, which is markedly terminal and the six nuclei of the lemnisci are distributed in the middle and hinder regions.

Specific Diagnosis

Oligacanthorhynchidae with elongate body, males reaching a length of 16 to 28 mm. and females 22 to 47 mm.; proboscis globular or very slightly elongated, 0.43 to 0.66 mm. in diameter, possessing six spinal rows with six relatively strong hooks in each row. First two hooks stouter than rest. Root of third hook always asymmetrically bent towards left, whereas root of fourth hook bent towards right with root of third hook overlapping it. Proboscis receptacle sac-shaped, 1.05 to 1.35 mm. long with brain situated at about the middle of its length. Lemnisci reaching up to first testis in male, with 5 to 6 nuclei in anterior and middle region, and 14 to 18 mm. long. Male genital organs in posterior half of body, testes of equal size, 1.6 to 2.4 mm. long. Cement glands eight in number, extending as a paired series for 2.9 to 4 mm. Bursa when extruded

1.32 mm. long, representing a closed hand. Embryos 0.095 to 0.108 mm. by 0.07 to 0.082 mm.

Type Host : *Canis adustus.*
Habitat : Intestine.
Locality : Northern Rhodesia.
Types : Department of Parasitology, London School of Hygiene and Tropical Medicine.

Summary

An account is given of the general morphology of *Echinopardalis lerouxi* n.sp., from the intestine of *Canis adustus*, in Northern Rhodesia. This new acanthocephalan agrees with the characters of the genus *Echinopardalis*, and differs from all the other known species included in this genus. The characteristic features of the species are enumerated.

References

Meyer, A., 1931.—"Neue Acanthocephalen aus dem Berliner Museum. Begrundung eines neuen Acanthocephalensystems auf grund einer untersuchung der Berliner sammlung." *Zool. Jahrb.* Bd.62, 53–108. (W.L.23831.)

———1932–33.—"Acanthocephala. In Bronn's Klassen und Ordnungen des Tierreichs." Bd.4, Abt.2, Buch 2. Leipzig. 582 pp. (W.L. 3592.)

Neveu-Lemaire, M., 1936.—"Traité d'Helminthologie Medicale et Vètèrinaire. Paris. 1330–1360.

Pujatti, D., 1951.—"Una nuova specie del genre *Echinopardalis* Travassos, 1918 rinvenuta in India." *Riv. Parassit.*, **12,** 119–128. (W.L. 19442a.)

Southwell, T. and Macfie, J. W. S., 1925.—"On a collection of Acanthocephala in the Liverpool School of Tropical Medicine." *Ann. Trop. Med. Parasit.* **19,** 141–184. (W.L. 1063.)

Travassos, L., 1917.—"Contribuicoes para o conhecimento da fauna helmintolojica brazileira. VI. Revisao dos acanthocephalos brazileiros. Parte 1. Fam. Gigantorhynchidae Hamann, 1872." *Mem. Inst. Osw. Cruz.* **9,** 5–62. (W.L. 13465.)

Van Cleave, H. J., 1945.—"The Acanthocephalan genus *Corynosoma*. I. The species found in water birds of North America." *J. Parasit.*, **31,** 332–340. (W.L. 11428.)

———1953.—"Acanthocephala of North American Mammals." *Illinois Biol. Monogr.* **23,** 1–179. (W.L. 9822.)

Van Cleave, H. J. and Ross, J. A., 1947.—"Use of Trisodium Phosphate in microscopical technic." *Science.* **106,** 194. (W.L. 19938.)

Witenberg, G., 1934.—"Parasitic worms of dogs and cats in Palestine." *Vet. Rec.* **14,** 232–238. (W.L. 22523.)

———1938.—"Studies on Acanthocephala. 3. Genus *Oncicola*." *Livro Jub. Prof. Travassos.* Rio de Janeiro. 537–560.

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Three New Species of the Genus *Neodiplostomum* Railliet, 1919, from Central African Birds of Prey, with a note on *Neodiplostomum canaliculatum* (Nicoll, 1914) Dubois, 1937.*

By B. Bisseru, M.Sc., Ph.D.

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

The genus *Neodiplostomum* Railliet, 1919 (syn. *Diplostomum* Brandes, 1888 (in part); *Conchogaster* Lutz, 1928; *Triplostomum* Lutz, 1928; *Neodiplostomoides* Vidyarthi, 1938) has the following generic diagnosis :—

Diplostomatini with oral sucker globular to ellipsoidal; body clearly divided by a more or less marked transverse constriction into two distinct regions, an anterior and a posterior one with the anterior body larger than the posterior body; cephalic extremity devoid of pseudosuckers or ear-like appendages; lateral margins of the forebody unite posteriorly to form a free overhanging margin; presence of an elliptical to circular holdfast organ, whose longitudinal diameter may reach from 1/7 to 4/7 of the length of the anterior segment; Mehlis' gland in the testicular zone; presence of a well developed bursa copulatrix which is not evaginable. Parasites of birds.

Type species: *Neodiplostomum spathulaeforme* (Brandes, 1888).

Dubois (1953) has replaced *Neodiplostomum spathulaeforme* (Brandes, 1888) as type of the genus *Neodiplostomum*, with *Neodiplostomum spathoides* Dubois, 1937, but this constitutes an infringement of Article 30(a) of the International Rules on Zoological Nomenclature since *N. spathulaeforme* has already been designated by Railliet (1919) and accepted as the genotype. Moreover, the assignment by Dubois (1953) of *N. spathulaeforme* as a "species delineatae" has no recognition in the International Rules.

In a comparison with the other known genera of the subsubfamily Diplostomatini Dubois, 1936, the species of *Neodiplostomum* may be found to agree in some respects with the genera *Diplostomum* v. Nordmann, 1832, *Bolbophorus* Dubois, 1934 and *Lophosicyadiplostomum* Dubois, 1936. Such generic differences as the presence of pseudosuckers on the sides of the forebody in *Diplostomum*, presence

* Part of a thesis approved by the University of London for the award of the Ph.D. degree.

of lateral suctorial pockets in *Bolbophorus*, the ellipsoidal or tri-radiate oral sucker with an annular equatorial crest, making a marked projection dorsally and laterally in the genus *Lophosicyadiplostomum*, would inhibit the inclusion of the present forms in the above three genera. The closely allied genera *Neodiplostomoides* Vidyarthi, 1938 which was separated from *Neodiplostomum* mainly on the presence of a hammer-shaped genital bulb, a feature doubted both by Dubois and Bhalerao (1942) is now a synonym of *Neodiplostomum*.

Dubois (1937) subdivided the genus *Neodiplostomum* Railliet, 1919 into two subgenera, *Neodiplostomum* Dubois, 1937, in which the hermaphrodite canal opens directly into the bursa copulatrix, without traversing a genital cone, with the anterior testis generally asymmetrically developed; and the subgenus *Conodiplostomum* Dubois, 1937, in which the hermaphrodite canal traverses a genital cone before opening into the bursa copulatrix, and in which both testes are generally symmetrically developed. In view of the above subdivision the species described in the following pages have been assigned to the subgenus *Neodiplostomum*, in that they principally lack a genital cone. Dubois (1953) in his mongraph lists 13 definite species in this subgenus, all these recognised forms parasitising avian hosts.

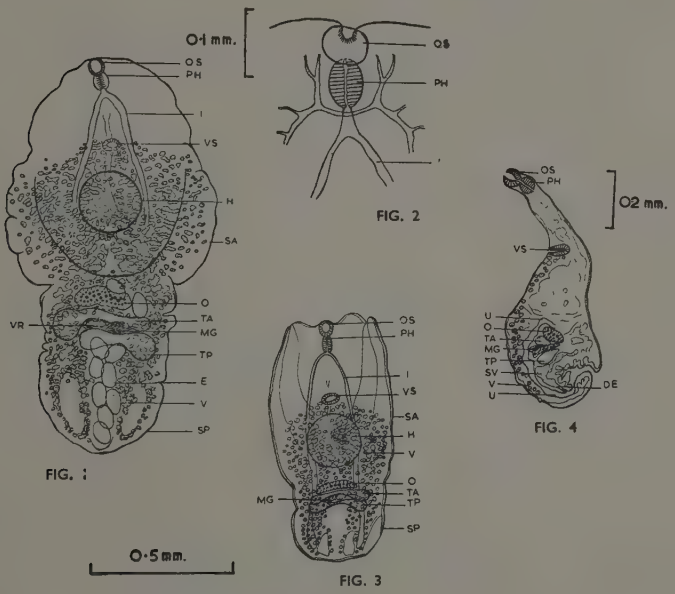
Diplostomatidae Poirier, 1886

Neodiplostomum (*Neodiplostomum*) *berghaani* n.sp.

Eight specimens of this trematode were collected from the small intestine of the Bateleur Eagle (Berghaan), *Terathopius ecaudata* in December, 1943, in Northern Rhodesia. The name *Neodiplostomum* (*N.*) *berghaani* n.sp. is proposed for this form, the name "Berghaan" being the Afrikaans equivalent for this bird in Southern Africa. Only two specimens possessed eggs.

The body is small, 0·86 to 1·43 mm. long and 0·315 to 0·435 mm. wide across the body constriction. The forebody, which is about one and a half to twice as long as the hindbody varying from being avoid to foliaceous, is 0·6 to 0·8 mm. long by 0·45 to 0·75 mm. wide across the holdfast organ. Oral sucker is subterminal, 0·054 to 0·06 mm. long by 0·057 to 0·064 mm. wide. The ventral sucker which is placed on an average of 39/100 of the length of the anterior segment, is 0·05 mm. long by 0·065 mm. wide. The pharynx measures 0·06 to 0·072 mm. longitudinally by 0·046 to 0·068 mm. transversely. The oesophagus is short, 0·018 to 0·04 mm. long, but it is practically absent in some specimens. The intestinal caeca are simple, terminating a short distance from the posterior end of the

body. The holdfast organ, in the posterior half of the forebody, is 0·2 mm. in diameter and circular in outline. The adhesive gland cells were not observed. No lateral suctorial cups or specialised glands are developed on the forebody. The transverse nerve cord measures 0·097 mm. across. The two anterior cephalic nerve cords divide into two on either side of the pharynx. The two posterior branches on either side of the oesophagus give off two lateral nerves and proceed posteriorly to enter the holdfast organ. Their course posterior to this organ has not been observed.



Neodiplostomum (*N.*) *berghaani* n.sp.

Fig. 1. Ventral view of worm.
Fig. 2. Anterior end, showing nerve cords.
Fig. 3. Ventral view of young individual.
Fig. 4. Longitudinal section of body. (Young individual).

Abbreviations used in Figs. 1--7.

AG = Adhesive gland. **B** = Bursa. **DE** = Ductus ejaculatorius. **E** = Egg. **I** = Intestinal caecum. **H** = Holdfast organ. **MG** = Mehlis' gland. **O** = Ovary. **OS** = Oral sucker. **PH** = Pharynx. **SA** = Anterior segment. **SP** = Posterior segment. **SV** = Seminal vesicle. **TA** = Anterior testis. **TP** = Posterior testis. **U** = Uterus. **V** = Vitellaria. **VR** = Vitelline reservoir. **VS** = Ventral sucker.

The hindbody is semi-cylindrical, 0·3 to 0·6 mm. long by 0·35 to 0·45 mm. wide, containing the bursa copulatrix on the dorsal side. The transversely elongate ovary is ovoid to rectangular, and measures 0·05 to 0·073 mm. longitudinally by 0·15 to 0·21 mm. transversely. It lies in front of the testes and is placed medianly at about the junction of the two body regions. The Mehlis' gland complex and vitelline reservoir are in the inter-testicular field. The Mehlis' gland complex is transversely elongate and slightly overlapped dorsally by the anterior testis. The uterus after emerging from the ootype proceeds anteriorly beyond the ovary for a short distance, and makes a dorso-ventral turn anterior to the ovary, proceeding in a sinuous course posteriorly to open at the genital pore. The eggs are 0·09 to 0·094 mm. long by 0·051 to 0·054 mm. wide. The two gravid individuals have two and ten eggs in their uteri respectively. The follicular vitellaria are distributed in both body segments, being grouped closely around the holdfast organ, and extending as far forward as the anterior border of the acetabulum in the first segment, and posterior to about the level of the genital pore in the hindbody. The follicles in the post-testicular region are in two bands, mainly disposed around the intestinal caeca.

The transversely elongate testes are tandem and close behind the ovary, and possess a median narrow isthmus in young individuals. Both testes are in the anterior half of the hindbody. The anterior border of the first testis is situated between the 18th to 24th hundredths of the length of the posterior segment. The transverse diameter of the first and second testes are 0·22 to 0·35 mm. and 0·27 to 0·29 mm. respectively. The anterior testis is asymmetrically developed. Seminal vesicle is convoluted and dilated in the area behind posterior testis and passes posteriorly almost parallel and dorsal to uterus, constricting to form the ejaculatory duct, which has a muscular wall, and joins the uterine duct just before its connection with the genital atrium. A genital cone is absent.

Systematic Relationships

This new trematode differs markedly from the other members of the subgenus *Neodiplostomum*. The present specimens have been compared with *N.* (*Neodiplostomum*) *hawkei*, a parasite of the common Indian Hawk, *Accipiter nisus melanoschistus*, but such characters as a circular holdfast organ, smaller eggs, disposition, nature and size of ovary and testes make the present examples distinct.

Of the two African species *N.* (*N.*) *cochleare* (Krause, 1914) LaRue, 1926 and *N. canaliculatum* (Nicoll, 1914) Dubois, 1937, the present specimens most resemble *N. canaliculatum* in the following features : (i) The ovoid anterior body and narrow posterior body. (ii) The general distribution of vitellaria in the body. (iii) Size of oesophagus. (iv) The ratio of the pharynx, oral sucker and ventral sucker to each other. (v) Position of the acetabulum in relation to the length of the anterior segment. But such differences as the small size of the present species which has a smaller oral sucker, pharynx and acetabulum, with a different ratio as regards the antero-posterior body length, a spherical holdfast organ instead of an ovoid one, size of testes, size of eggs and the anterior limit of the vitelline follicles from the anterior extremity of the body which is 0·53 mm. in *N. canaliculatum* and 0·3 mm. in *N.* (*N.*) *berghaani* n.sp., separates these two forms.

Host : *Terathopius ecaudata.*

Habitat : Small intestine.

Locality : N. Rhodesia.

Types : Department of Parasitology, London School of Hygiene and Tropical Medicine.

Neodiplostomum (*Neodiplostomum*) *prudhoei* n.sp.

The Cape Sea-Eagle, *Cuncuma vocifer vocifer* harboured a number of this new species, the parasites being in varying states of expansion and contraction. The total length of the body is 1·38 to 2·15 mm.; width at antero-posterior body constriction is 0·32 to 0·42 mm. The anterior segment is leaf-like and spoon-shaped with a more or less pointed anterior end, which is absent in some forms, and is occupied by the oral sucker, 0·065 to 0·09 mm. long by 0·95 to 0·072 mm. wide. The forebody measures 1·36 to 1·41 mm. long by 0·58 to 0·66 mm. wide, with its lateral margin curved ventrally, sometimes overlapping the lateral edges of the holdfast organ. The forebody is about twice as long as the more or less cylindrical hindbody, 0·61 to 0·74 mm. long by 0·42 to 0·48 mm. wide. A very short prepharynx 0·016 mm. long is observed in some specimens. The pharynx measures 0·095 to 0·108 mm. long by 0·05 to 0·07 mm. wide. The oesophagus is short, 0·03 to 0·04 mm. long. The intestinal caeca reach almost to the posterior end of the body. The ventral sucker whose distance varies from 0·58 to 0·68 mm. from the anterior extremity of the body, has a circular to ovoidal appearance 0·08 to 0·092 mm. long by 0·09 to 0·116 mm. broad. It is situated about midway the length of the anterior segment and is sometimes very

slightly anteriorly placed, its posterior border being about 0·02 to 0·15 mm. from the anterior border of the holdfast organ, which is placed in the posterior half of the forebody and measures 0·42 to 0·48 mm. long by 0·24 to 0·26 mm. broad. A compact, rounded, adhesive gland mass is present on the postero-dorsal surface of the holdfast organ.

The testes are transversely elongate, the anterior one being asymmetrically developed. The anterior and posterior testes have a transverse diameter of 0·28 to 0·33 mm. and 0·32 to 0·37 mm. respectively. The seminal vesicle is coiled. The copulatory bursa is spacious, in the posterior third of the hind segment. The hermaphrodite canal opens dorsally near the end of the body. A genital cone is absent.

The ovary is transversely elongate, 0·13 mm. long by 0·19 mm. to 0·28 mm. wide and is placed at about the junction of the two body segments or very slightly posterior. Mehlis' gland complex is to the right of the median line. The vitelline follicles are well developed, and extend usually beyond and in some form stops at the level of the acetabulum. The follicles are gathered in the holdfast organ, where they appear compacted and larger, and lie ventrally in the posterior body, being more laterally disposed in the post-testicular area. The vitelline reservoir is median and inter-testicular. The eggs are 0·08 to 0·097 mm. long by 0·052 to 0·063 mm. wide.

Systematic Relationships

The species, *Neodiplostomum* (*N.*) *cochleare* was first described by Krause (1914) from *Bubo bubo ascalaphus* in Dongola, Sudan, Africa. Other hosts of this parasite, which has been described from such wide areas as Japan, Egypt and the United States, are *Bubo virginianus*, *Nyctea nyctea* and *Milvus migrans aegyptius*. Dubois (1938) distinguished two varieties, viz., *N. cochleare japonicum* Yamaguti, 1935 and *N. cochleare calaophilum* Verma, 1936, as being distinct from the typical *cochleare*. *N. cochleare japonicum*, described from *Asio otus otus*, is separated from the typical form by its possession of a smaller pharynx, in comparison with the oral sucker; very much larger ventral sucker; ovary in first quarter of the posterior segment, and by its smaller anterior testis. *N. cochleare calaophilum* from *Dichoceros bicornis* has been separated on account of its host, which is a hornbill instead of a falconiform bird and by its possession of much larger eggs, 0·1008 × 0·08 mm., instead of the typical *cochleare* egg which is 0·086 to 0·096 mm. × 0·052 to 0·063 mm. Dubois (1944) raised this latter variety to the status of a distinct species *N. calaophilum* but in 1953 regarded it

as a *species inquirendae.* Similarly the variety *N. cochleare var. americanum* Chandler and Rausch, 1947 was referred to as *N.* (*Neodiplostomum*) *americanum.*

(*N.* (*N.*) *cochleare* seems to parasitize a wide range of birds of prey, and is probably world wide in its distribution. The structural modifications of *N.* (*N.*) *cochleare* from host to host may indicate that with further change or mutation its different varieties may further develop into distinct species, since the type of the host parasitized provides an isolating medium, each host being restricted in its food habits and differing in its physiological conditions.)

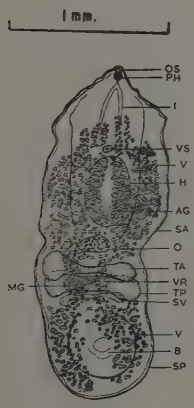


FIG. 5

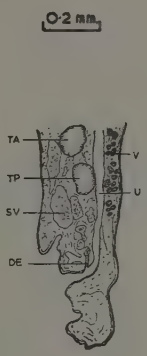


FIG. 6

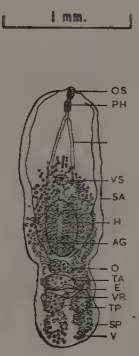


FIG. 7

Neodiplostomum (*N.*) *pseudogypsis* n.sp.
Fig. 5. Ventral view of worm.
Fig. 6. Longitudinal section of posterior end showing genital ducts.
Neodiplostomum (*N.*) *prudhoei* n.sp.
Fig. 7. Ventral view of worm.

The present specimens differ from *N. calaophilum* and *N.* (*N.*) *americanum* in possessing smaller eggs. It is separated from *N. cochleare var. japonicum* in its smaller size, small ventral sucker and larger pharynx. A comparison with the European form, *Neodiplostomum* (*N.*) *spathoides* Dubois, 1937 reveals such differences as the distribution of the vitellaria, nature of the bursa copulatrix, ovary and testes, size of the eggs and the fact that *N.* (*N.*) *spathoides* is a much smaller form.

The form from *Cuncuma vocifer vocifer* is here regarded as a distinct species, as it differs from *N.* (*N.*) *cochleare* in being smaller

in size, with small testes which are far removed from the lateral walls of the body, presence of a short prepharynx, large pharynx and a more narrowly ellipsoidal holdfast organ. This new species is called *Neodiplostomum* (*Neodiplostomum*) *prudhoei* n.sp.

Host : *Cuncuma vocifer vocifer*.
Habitat : Intestine.
Locality : Northern Rhodesia.
Types : Department of Parasitology, London School of Hygiene and Tropical Medicine.

Neodiplostomum (*Neodiplostomum*) *pseudogypsis* n.sp.

Two specimens were obtained from the intestine of the white-backed vulture, *Pseudogyps africanus*, in Northern Rhodesia.

The body measures 2·1 mm. long, with a breadth of 0·6 mm. at the body constriction. The forebody, 1·17 mm. long by 0·77 mm. wide, has its anterior extremity pointed with the lateral edges turned over ventrally and uniting with each other behind the holdfast organ. The hindbody, 0·94 mm. long by 0·675 mm. wide, carries the reproductive organs and the spacious, well developed bursa copulatrix, 0·42 mm. long by 0·395 mm. wide.

Oral sucker is terminal, 0·06 mm. long by 0·05 mm. wide. A prepharynx is absent. The pharynx is slightly larger than the oral sucker. The oesophagus is short and intestinal caeca are narrow in the forebody, but become somewhat wider posteriorly terminating a small distance from the posterior end of the body. The acetabulum is small and weak, 0·045 mm. by 0·068 mm. and is situated at about 44/100 of the length of the anterior segment, being not far removed from the anterior border of the holdfast organ. The holdfast organ is elliptical, 0·405 mm. long by 0·27 mm. wide, and is placed in the posterior two-thirds of the forebody, with a median longitudinal opening or groove and a rounded mass of adhesive gland cells which lie dorso-posteriorly.

Testes are tandem, the posterior testis being larger than the anterior one. Both testes are found in the anterior half of the hindbody, and have their lateral lobes joined by a narrow isthmus, the lobes of the anterior testis which is asymmetrically developed overlapping to a small degree the corresponding lobes of the posterior testis. The transverse diameters of the first and second testes are 0·58 mm. and 0·62 mm. respectively. The vesicula seminalis is much convoluted and placed immediately behind the second testis. The genital pore is fairly wide, dorsal and near the posterior end of the body. A genital cone is absent.

The ovary is ovoid, with a transverse diameter of 0·18 mm. and

is placed almost at the point of the body constriction. The Mehlis' gland complex and vitelline reservoir are both ventral to the posterior testis, the former on the left and the latter on the right of the median line of the body. The uterus reaches anteriorly to the junction between the anterior and posterior body regions, but does not penetrate the region of the holdfast organ. It then bends posteriorly, pursuing a more or less straight course, leading to the common genital pore. Vitellaria are well developed in both body divisions. In the forebody the vitelline follicles which are grouped closer towards the holdfast organ, fade out gradually anterior to the ventral sucker. Up to about the first half of the hindbody the follicles are centrally disposed in two ribbons which merge in the post-testicular region, but these follicles are immediately separated in two laterally disposed sheets, which extend almost to the end of the body. Eggs are absent in the two specimens.

Systematic Relationships

The previously known forms have been compared with the present specimens, which differ from all these hitherto described forms. The species that has been brought into close comparison is *N.* (*N.*) *cochleare* (Krause, 1914) LaRue, 1926 from owls in Egypt and the United States, but this species is obviously far removed and differs in the ratio and size of the antero-posterior body, both as regards length and breadth. Other differences are seen in the size and nature of the testes, nature of bursa copulatrix, distribution of vitellaria, position of Mehlis' gland and vitelline reservoir and the size and relation to each other of the oral sucker, pharynx and acetabulum.

N. canaliculatum (Nicoll, 1914) Dubois, 1937 from *Bubo bubo ascalaphus*, in Egypt differs from the present forms in the ratio of the body size, length and breadth of the oral sucker, pharynx and acetabulum, and in the general morphology of the body.

This species differs from the two previously described forms, *N.* (*N.*) *berghaani* and *N.* (*N.*) *prudhoei* in having a much larger pharynx in comparison with the oral sucker and acetabulum, nature and size of the ovary and testes, the very spacious bursa copulatrix and the extent and distribution of the vitellaria in both body segments.

Host: *Pseudogyps africanus.*
Habitat: Intestine.
Locality: Northern Rhodesia.
Type: Department of Parasitology, London School of Hygiene and Tropical Medicine.

Neodiplostomum canaliculatum (Nicoll, 1914) Dubois, 1937

(Syn.: *Hemistomum canaliculatum* Nicoll, 1914)

The writer has had the opportunity of examining the type specimen *Hemistomum canaliculatum=Neodiplostomum canaliculatum* (Type No. 278), deposited in the Parasitology Department, London School of Hygiene and Tropical Medicine. This trematode was obtained from the intestine of the Egyptian Eagle-owl, *Bubo ascalaphus*, which died in the Zoological Society Gardens, London, on the 24.10.1911.

Dubois (1953) segregated *N. canaliculatum* (Nicoll, 1914), as representing a *species inquirendae*, but a careful examination of the available type specimen by the writer fails to find any reasons for supporting this action. This species fits the characterization of the genus *Neodiplostomum* as given by Dubois (1938, 1953), and this evidence shows conclusively how strong are the grounds for the recognition of *N. canaliculatum* in the genus *Neodiplostomum*. In his comprehensive Monograph on the Strigeid Trematodes, Dubois (1938 : 228) granted recognition to the features of *Hemistomum canaliculatum* Nicoll, 1914 and expressed confidence in its generic assignment, referring this species to *Neodiplostomum*,—"C'est ainsi que nous pouvons attribuer avec certitude cette espèce au genre *Neodiplostomum* et écarter les doutes résultant d'une observation faite par Nicoll (1914a)",—basing his evidence on Nicoll's original description and a microphotograph of the type specimen received from the London School of Hygiene and Tropical Medicine.

Nicoll (1914) probably made an error in observation by stating that "The lateral glandular pits are not very well marked". On the basis of an examination of the present type specimen of *N. canaliculatum*, lateral glandular pits are definitely absent. The body is 2·55 mm. long, with anterior body longer than posterior one, in ratio of 10 : 7. The forebody measures 1·5 mm. long by 1·2 mm. wide across the holdfast organ and the hindbody is 1·05 mm. long by 0·705 mm. wide in post-testicular area. The oral sucker is 0·075 mm. by 0·09 mm.; pharynx 0·095 mm. by 0·065 mm.; ventral sucker 0·092 mm. in diameter, and situated at 38th hundredths (0·57 mm. from anterior extremity of body) of the length of anterior segment or at a position of 2/9 of the total body length. The oesophagus is 0·03 mm. long. Holdfast organ is ovoid, 0·43 mm. long by 0·32 mm. wide. The transverse diameters of the ovary, anterior and posterior testes are 0·2 mm., 0·38 mm., and 0·51 mm. respectively. Anterior testis is asymmetrically developed, and only

the ovary and posterior testis could be described as "reniform" (see Nicoll, 1914). Eggs are 0·1 to 0·108 mm. long by 0·06 to 0·067 mm. wide. The vitelline follicles reach slightly anterior to acetabulum (0·53 mm. from front end of body). They are more concentrated in the region of the holdfast organ and reach up to the caudal end of the hindbody, in two main ventral bands.

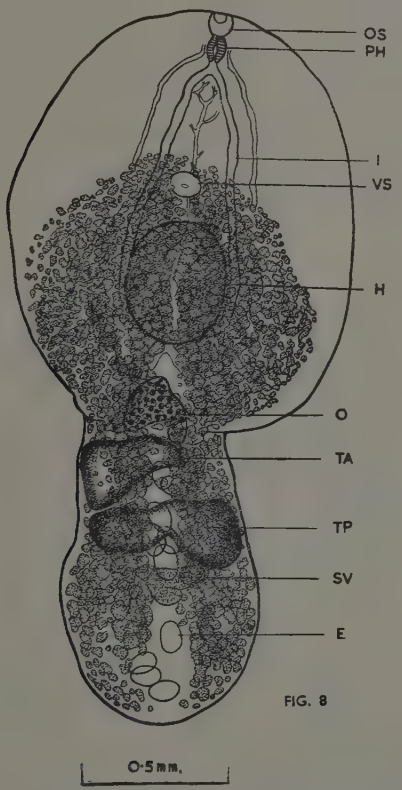


Fig. 8. *Neodiplostomum canaliculatum* (Nicoll, 1914) Dubois, 1937.

E = Egg. **H** = Holdfast organ. **I** = Intestinal caecum. **O** = Ovary. **PH** = Pharynx. **OS** = Oral sucker. **SV** = Seminal vesicle. **TA** = Anterior testis. **TP** = Posterior testis. **VS** = Ventral sucker.

Summary

Three new species of the genus *Neodiplostomum* Railliet, 1919 (Subgenus *Neodiplostomum*, Dubois, 1937) are described from avian hosts in Central Africa. They are *N.* (*N.*) *berghaani* from *Terathopius ecaudata*, *N.* (*N.*) *prudhoei* from *Cuncuma vocifer vocifer* and *N.* (*N.*) *pseudogypsis* from *Pseudogyps africanus*.

A note is added on *Neodiplostomum canaliculatum* (Nicoll, 1914) Dubois, 1937.

Acknowledgments

The trematodes discussed in the present paper formed part of a collection of parasitic worms, collected by Dr. P. L. LeRoux in Northern Rhodesia, in 1943. The writer is deeply indebted to him both for the material and for his patient criticism.

References

Brandes, G., 1888.—"Ueber das genus *Holostomum* Nitzsch (Vorlaufige Mittheilung)". *Zool. Anz.*, **11**, 424–426. (W.L. 23833).

Bhalerao, G. D., 1942.—"On Strigeida (Trematoda) from India." *Rec. Indian Mus.*, **44**, 207–216. (W.L. 17746).

Chandler, A. C. and Rausch, R., 1947.—"A study of Strigeids from Owls in North Central United States." *Trans. Amer. micr. Soc.*, **66**, 283–292. (W.L. 21400).

Dubois, G., 1937.—"Sur quelques Strigeides." *Rev. suisse Zool.*, **44**, 391–396. (W.L. 19288.)

———1938.—"Monographie des Strigeida (Trematoda)." *Mem. Soc. neuchâtel Sci. nat.*, **6**, 535pp. (W.L. 13318a.)

———1944.—"À propos de la Specificité parasitaire des Strigeida." *Bull. Soc. neuchâtel Sci. nat.*, **69**, 1–103. (W.L. 5299.)

———1953.—"Systematique des Strigeida, complement de la Monographie." *Mem. Soc. neuchâtel Sci. nat.*, **8**, 141 pp. (W.L.13318a.)

Krause, R., 1914.—"Beitrag zur Kenntnis der Hemistominen." *Z. wiss. Zool.*, **112**, 93–238. (W.L. 23635.)

Nicoll, W., 1914.—"Trematode Parasites from Animals dying in the Zoological Society's Gardens during 1911–1912." *Proc. zool. Soc. Lond.* (1), 139–154. (W.L. 16969b.)

Verma, S. C., 1936.—"Notes on Trematode Parasites of Indian Birds. Part I." *Allahabad Univ. Stud.* **12**, 147–188. (W.L. 393c.)

Vidyarthi, R. D., 1938.—"Avian trematodes of the genera *Neodiplostomoides* nov.gen. *Bolbophorus* Dubois, 1934, and *Glossodiplostomum* Dubois, 1932." *Parasitology*, **30**, 33–39. (W.L. 16035.)

Journal of Helminthology. Vol. XXX, No. 1, 1956, pp. 63–79.

On Four New Trematodes of the Genus *Strigea* from Central African Birds of Prey*

By B. Bisseru, M.Sc., Ph.D.

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

The trematodes described in this paper are assigned to the genus *Strigea* Abildgaard 1790 as outlined by Dubois (1938, 1953). The genus is characterized as follows :—

Strigeini : digestive system with pharynx ; presence of an ovoid anterior segment, which is cup-shaped without lateral expansions ; the vitelline follicles are distributed in the walls of the body as well as in the lobes of the holdfast organ ; posterior segment is without a well differentiated neck ; the copulatory bursa with a terminal pore is usually not externally well defined, the walls of the bursa being provided at the base with a muscular ring, which is well developed ; genital cone is clearly defined from the parenchyma by its own musculature and is traversed by the hermaphrodite canal which arises from the confluence at about the first third of its length, of the uterus and ductus ejaculatorius ; cirrus and cirrus pouch absent.

Type species : *Strigea strigis* (Schrank, 1788).

The genus *Apharyngostrigea* Ciurea, 1927, is closely related to *Strigea*, but differs in the absence of a pharynx. The genus *Ridgewayiella* Verma, 1936, erected to contain a single species *Ridgewayiella rarnai* Verma, 1936 from the night heron, *Nycticorax nycticorax* in India, mainly on "a very peculiar and characteristic muscular holdfast organ, twisted upon itself in a prominent fashion like a broad ridge", is synonymous with the genus *Apharyngostrigea* according to Vidyarthi (1937).

*Part of a thesis approved by the University of London for the award of the Ph.D. degree.

The genus *Strigea* and *Parastrigea* Szidat, 1928 also seem very closely related, the major points separating these two genera as indicated by Dubois being as follows :—

(I) In *Parastrigea* the vitelline follicles or glands which are distributed in both segments as in *Strigea*, show a tendency to concentrate in the anterior segment into two symmetrical, ovoid or sinuous masses, continued in two lateral projections of the holdfast organ, and are separated by a more or less deep furrow.

(II) The close proximity of the suckers which are relatively poorly developed, the acetabulum being compressed forwards to one-third or even one-quarter of the length of the segment, whose anterior opening is typically narrow.

On the other hand :—

(I) In the genus *Strigea* the vitelline follicles extend into the lips of the holdfast organ, without being concentrated in laterally expanded symmetrical masses.

(II) The suckers are not close together, the acetabulum being situated in the middle or even behind the middle of the anterior segment, the aperture of the segment being most often wide.

According to Dubois (1953), the genus *Strigea* contains twenty-two species. *Cotylurus streptocorpus* Verma, 1936, from the Indian fishing eagle, *Haliaeetus leucoryphus*, had been referred to *Strigea streptocorpus* by Dubois (1938), on account of the extent of the vitelline follicles into the anterior segment and the presence of a small ventral sucker in comparison with the oral. Dubois (1953) rejects *S. streptocorpus* as a valid species, as the very brief description without an illustration contradicts Article 25, (Proviso C, Section 1), of the International Rules on Zoological Nomenclature. The species *Strigea elongata* Yamaguti, 1935 from *Accipiter virgatus gularis* in Japan contains a single variety *S. elongata* var. *indica* Verma, 1936 from *Oriolus melanocephalus*, the description of the variety being based on a single specimen. It is separated from the type *S. elongata* on the smaller size of the body and the anterior testis is larger than the posterior one. The species *S. falconis* has four varieties namely, var. *brasiliana* Szidat, 1929 ; var. *eaglesa* Verma, 1936 ; var. *japonensis* Yamaguti, 1939 and var. *meleagris* Harwood, 1931 which is from a turkey. Dubois (1944) does not consider var. *meleagris* in his table which includes those forms from birds of prey and not

domestic animals which according to him have been subjected to abnormal infestations, as also in the case of the dove, *Streptopelia chinensis* harbouring *Strigea falconis*. Dubois (1953) does not consider the varieties *S. falconis japonensis* Yamaguti, 1939 and *S. falconis eaglesa* Verma, 1936, as according to him they are not distinguishable from the type *Strigea falconis* Szidat, 1928.

Dubois in his survey of the species in the genus *Strigea* apparently overlooks *Strigea pelagidis* Dubinin, 1938, from *Pelagidis falcinellus*. The more recent additions to this genus are all North American forms. They are *Strigea elegans* Chandler and Rausch, 1947, from *Bubo virginianus virginianus* ; *Strigea macroconophora* Dubois and Rausch, 1948 : 1950, from *Buteo jamaicaensis borealis* and *Strigea sphaerula macrosicya* Dubois and Rausch, 1950, from *Corvus corax principalis*.

STRIGEA NEOTIDIS N.SP.

About two dozen specimens of *Strigea neotidis* n.sp. were obtained from the small intestine of *Neotis cafra denhami* in Northern Rhodesia. In the fixed state the trematodes show a typical ventral convexity. The longitudinal axis of the body is flexed in such a way that the worm has a more or less crescentic or sickle-shaped appearance. The strong curvature of the posterior body, especially the post-ovarian region is very characteristic in all the specimens and makes nearly a right angle with the pre-ovarian part.

The total length of the worm varies between 1.9 to 2.6 mm. The roughly spheroidal anterior segment, 0.87 to 1.09 mm. long and 1.03 to 1.05 mm. wide, is cup-shaped, with the latero-ventral lobes of the holdfast organ extending forward from the base of the sides of the cup. The broad lobes of the holdfast organ do not seem to project to any appreciable extent from the wide, anterior opening of the cup. The anterior segment narrows abruptly leading into the narrower cylindrical posterior segment, which is so connected to the base of the anterior segment, that a clearly defined transverse constriction is lacking. The hindbody, about 1 to 1.46 mm. long and 0.45 to 0.5 mm. wide, is reduced to 0.27 to 0.29 mm. in width at the level of the bursa copulatrix. The ratio of the maximum length of the posterior body to the anterior body varies from 1.13 in contracted specimens to 1.56 in extended forms.

The oral sucker is situated far forward in the cup formed by the

first segment and lies centrally between two slight blunt prolongations of the dorsal wall of the cup. It measures 0.129 to 0.14 mm. by 0.13 to 0.16 mm., being almost spherical, whereas the pharynx, which is contiguous with the oral sucker, has been seen with difficulty in some *in toto* preparations, being largely hidden by the lobes of the holdfast organ. Moreover the muscle fibres of the pharynx are weakly developed. It measures 0.079 mm. long by 0.058 mm. wide. The dimensions of the ventral sucker are 0.228 to 0.264 mm. by 0.216 to 0.245 mm. The lobulate adhesive gland is 0.172 to 0.223 mm. long by 0.176 to 0.198 mm. wide. It is situated at a distance of 0.028 to 0.046 mm. behind the anterior border of the acetabulum, and reaches almost to the body division.

The ovary, situated between the 38th to 44th hundredths of the length of the posterior segment, is about 0.083 mm. in antero-posterior diameter and 0.208 to 0.24 mm. in transverse diameter. The Mehlis' gland complex lies between the testes. On emerging from the ootype the uterus proceeds anteriorly and reaches to a distance of about 0.4 mm. anterior to the ovary and about 0.075 mm. posterior to the junction of the anterior and posterior body segments.

The muscular genital cone, which receives the terminal male and female ducts, has a diameter of 0.09 mm. in the region of the sucker-like genital ring (Ringnapf of German writers), which has a width of 0.038 mm. The genital cone projects into the bursa which is 0.108 mm. wide and 0.156 mm. deep.

The vitellaria occupy both body segments, the follicles being most dense in the anterior half of the posterior body. In the anterior segment the follicles extend into the lobes of the holdfast organ, reaching anteriorly up to the oral sucker. In the posterior body the vitellaria occupy the ventral part and penetrate the wall of the bursa, ending at a distance of 0.072 to 0.108 mm. from the posterior extremity of the body. The vitelline reservoir is situated between the testes, and is transversely elongate, being overlapped dorsally by the posterior testis in the majority of specimens.

Both anterior and posterior testis are more or less dumbell shaped lying in the posterior half of the hindbody, with the large lateral lobes joined by an isthmus which measures 0.058 mm. and 0.043 mm. in longitudinal diameter in the first and second testis respectively. The transverse diameter of the anterior testis is 0.34 to 0.4 mm.; the right lateral lobe measures 0.15 mm. and the

left lateral lobe is 0.086 to 0.144 mm. in antero-posterior diameter. The corresponding measurements of the posterior testis, which is larger than the anterior one are 0.38 to 0.432 mm.; 0.13 to 0.158 mm. and 0.125 to 0.24 mm. A seminal reservoir is clearly distinguishable between the ovary and first testis and the vesicula seminalis is much convoluted on the postero-dorsal side of the hind testis. A paraprostate is absent.

The eggs are 0.115 to 0.13 mm. long by 0.061 to 0.068 mm. wide.

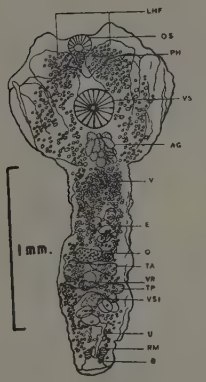


FIG. 1

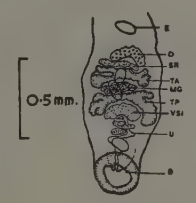


FIG. 2

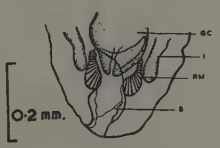


FIG. 3

FIG. 4

Strigea neotidis n.sp.

Fig. 1.—Ventral view. Fig. 2.—Posterior end of body, showing disposition of male and female genitalia. Fig. 3.—Posterior extremity of body, showing bursa copulatorix and genital cone. Fig. 4.—Latero-ventral view.

Abbreviations used in Figs. 1–4.

AG = Adhesive gland; **B** = Bursa; **E** = Egg; **GC** = Genital cone; **I** = Intestinal caecum; **LHF** = Lobes of holdfast organ; **MG** = Mehlis' gland; **O** = Ovary; **OES** = Oesophagus; **OS** = Oral sucker; **PH** = Pharynx; **RM** = Ring muscle; **SR** = Seminal reservoir; **TA** = Anterior testis; **TP** = Posterior testis; **U** = Uterus; **V** = Vitellaria; **VS** = Ventral sucker; **VSI** = Vesicula seminalis; **VR** = Vitelline reservoir.

Relationships

This trematode, in having the vitelline follicles penetrating the wall of the bursa and extending almost to the hind end of the body, may be separated from the following species of the genus *Strigea*,

namely, *nugax, glandulosa, promiscua, mcgregori, elliptica, elongata, falconis, globocephala, elegans, nephronis* and *orientalis.* Of the remaining members of this genus the present species is perhaps adequately distinguished by the very characteristic laterally expanded forebody and much narrower hindbody ; larger dimensions of the eggs ; the very weakly muscular pharynx ; large ventral sucker ; the posterior position of the ovary in the hindbody ; the relatively small size of the testes in relation to the size of the posterior body and the nature of the bursa copulatrix and genital cone.

In the nature of the ovary and lobed testes it may be likened to *S. nephronis* Vidyarthi, 1937, but differs from it in many other respects. The species is recorded as new under the name *Strigea neotidis* n.sp.

Host : *Neotis cafra denhami.*

Habitat : Small intestine.

Locality : Northern Rhodesia.

Types and Co-types : Department of Parasitology, London School of Hygiene and Tropical Medicine.

Strigea lilensis n.sp.

This trematode was collected from the small intestine of *Pseudogyps africanus* in Northern Rhodesia. The material comprises a single worm, measuring 4.12 mm. long. All of its morphological features necessary for taxonomic study, have been clearly determined.

The body of the sexually mature parasite is arched, with the anterior and posterior extremities bluntly truncated. The body is uniformly covered with an aspinose cuticle closely applied to a paler staining subcuticle, which is clearly seen in parts where the cuticle proper is partly missing. The compact forebody measures 1.42 mm. in length by 1.29 mm. in width. It unites almost imperceptibly, there being little or no signs of a transverse constriction, with a fairly thick hindbody. The hindbody is 2.7 mm. long and is about twice the length of the forebody. The transverse diameter which is less than that of the forebody, is about 1 mm. in the anterior testicular region and 0.57 mm. across the bursa copulatrix. Strong longitudinal muscle fibres originating in the posterior half of the

anterior body extend into the hindbody, the farthest extension of some fibres being up to the bursa copulatrix.

The oral sucker is 0.195 mm. long by 0.166 mm. wide. It leads into a globular, muscular pharynx, which has a diameter of 0.14 mm. The ventral sucker is situated at about the middle of the dorsal

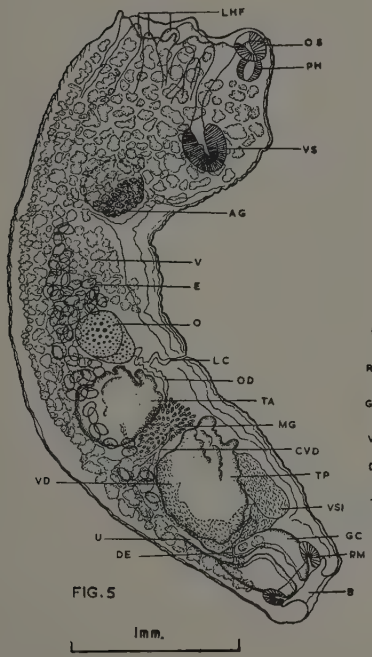


FIG. 5

Strigea lilensis n.sp.

Fig. 5.—Lateral view of entire worm.

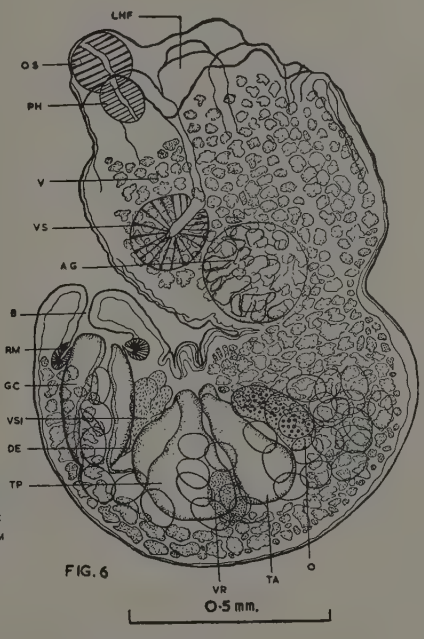


FIG. 6

Strigea cuncumae n.sp.

Fig. 6.—Lateral view of entire worm.

Abbreviations used in Figs. 5–6.

AG = Adhesive gland ; **B** = Bursa ; **CVD** = Common vitelline duct ; **DE** = Ductus ejaculatorius ; **E** = Egg ; **GC** = Genital cone ; **LHF** = Lobes of holdfast organ ; **LC** = Laurer's canal ; **MG** = Mehlis' gland ; **O** = Ovary ; **OD** = Oviduct ; **OS** = Oral sucker ; **PH** = Pharynx ; **RM** = Ring muscle ; **TA** = Anterior testis ; **TP** = Posterior testis ; **U** = Uterus ; **V** = Vitellaria ; **VD** = Vas deferens ; **VR** = Vitelline reservoir ; **VS** = Ventral sucker ; **VSI** = Vesicula seminalis.

portion of the forebody, and measures 0.29 mm. long by 0.245 mm. wide. The relatively small lobes of the holdfast organ project very slightly over the anterior rim of the forebody. The lobulate adhesive gland is almost circular, with a transverse diameter of 0.315 mm., and is situated close to the posterior border of the anterior segment of the body.

The ovary, placed close in front of the anterior testis, between the 20th to 34th hundredths of the length of the posterior body, measures 0.39 mm. long by 0.3 mm. wide. The concavity of the ovary is directed postero-dorsally, its anterior border being recurved into an arch directed ventro-anteriorly. The Mehlis' gland complex measures 0.27 mm. long by 0.45 mm. wide. It is placed more or less equatorially between the two testes in the posterior body. The oviduct arises from the dorso-posterior border of the ovary, being slightly coiled, and passing dorsally to the anterior testis without overlapping it to any appreciable extent. In the pre-ovarian region the uterus almost reaches the antero-posterior body division. The loops of the uterus are much dilated and on entering the genital cone unite with the ejaculatory duct to open into the genital atrium within the bursa copulatrix. The eggs are fairly numerous. They are oval, with thin shells. Their measurements vary from 0.1 to 0.12 mm. long by 0.058 to 0.065 mm. wide.

The vitellaria, which extend profusely up to the pharynx in the forebody, show their heaviest concentration in the pre-ovarian part of the hindbody. Posterior to the ovary the follicles are confined largely to the median ventral field, ventral to the testes and Mehlis' gland complex, but behind the posterior testis the follicles are found scattered laterally in the region of the vesicula seminalis. The follicles penetrate the walls of the genital cone.

The anterior testis is 0.42 mm. long by 0.45 mm. wide. The corresponding measurements of the posterior testis are 0.56 mm. long by 0.59 mm. wide. The first testis is placed more in the middle third of the hindbody, while the posterior one lies at the junction of the middle and last third of the hindbody. The testicular lobes are directed largely towards the dorsal surface of the body. Vasa efferentia arising from each testis are dilated with sperms. Vesicula seminalis is coiled immediately behind the hinder testis, and leads into a narrow ejaculatory duct. A paraprostate is absent. The genital cone measures 0.495 mm. long by 0.4 mm. wide. The bursa copulatrix is 0.38 mm. in diameter, at the level of the muscular ring, with a terminal aperture and depth of about 0.18 mm.

Relationships

This species, in which the vitelline follicles do not extend to the walls of copulatory bursa, or reach the hind end of the body, differs from the following members of the genus *Strigea*:—*bulbosa*, *vaginata*, *strigis*, *infundibuliformis*, *flosculus*, *sphaerocephala*, *intermedia*, *sphaerula*, *nicolli*, *baylisi*, *suttoni* and *macroconophora*. It approaches *S. elongata* Yamaguti, 1935 in the length of the body and in the position of the adhesive gland. The distribution of the vitellaria compares closely with the Japanese species, but the present species differs from it in the size and situation of the ovary which is 40th to 47th hundredths of the length of the posterior segment in the typical *elongata*, and 20th to 34th hundredths in the present species. Further, the most striking differences from *S. elongata* are evident in the size and position of the testes, Mehlis' gland, seminal vesicle, bursa copulatrix and genital cone. The ventral sucker is larger with a greater distance between it and the adhesive gland. Moreover, the oral sucker, pharynx and eggs are larger in the present species, and the ratio of the posterior to the anterior body, is 3.83 to 4.14 for *S. elongata* and 2.03 for our species. The present species is therefore considered to be a form hitherto undescribed and represents a new species, called *Strigea lilensis* n.sp.

Host : *Pseudogyps africanus*.

Habitat : Small intestine.

Locality : N. Rhodesia.

Type : Department of Parasitology, London School of Hygiene and Tropical Medicine.

Strigea cuncumae n.sp.

This description of *Strigea cuncumae* n.sp. is based on two examples collected from the intestine of the Fish Eagle, *Cuncuma vocifer vocifer*, in N. Rhodesia.

The body which is ovoid in the preserved state is distinctly constricted into two portions and measures 1.5 to 1.9 mm. in total length, with a diameter of about 0.315 mm. across the bursa copulatrix. The dimensions of the anterior portion or forebody are 0.6 to 0.86 mm. long by 0.62 to 0.78 mm. wide. The terminal oral

sucker is 0.126 mm. long by 0.169 mm. wide. The pharynx is 0.115 mm. long by 0.097 mm. wide. The acetabulum measures 0.165 mm. in length by 0.26 mm. in width. The adhesive gland situated behind the acetabulum has a diameter of about 0.22 mm. It is made up of lobes which stain deeply in Acetic alum carmine. The lobes of the holdfast organ are well developed. The hindbody which is conspicuously arched forming almost a semicircle, measures 0.9 to 1.04 mm. in length by 0.56 to 0.6 mm. in width. The ratio of the length of the second portion or hindbody to the first is about 1.2. Strong dorso-lateral muscle fibres originating in the forebody extend into the hindbody up to the genital cone.

The testes, which are placed immediately posterior to the ovary, occupy the middle third of the hindbody. The anterior testis is 0.1 to 0.23 mm. long by 0.234 to 0.26 mm. wide, and the posterior testis is 0.18 to 0.25 mm. long by 0.27 mm. wide. Both testes appear deeply concave, the concavity facing dorsally, and the ventral border is convex. The seminal vesicle is much convoluted on the postero-dorsal side of the last testis. The ejaculatory duct descends ventrally to enter the uterus in the midst of the genital cone, which measures 0.338 mm. long by 0.18 mm. wide. A prostate gland is absent.

The ovary, 0.108 to 0.126 mm. long by 0.144 to 0.216 mm. wide is situated between the 21st to 25th hundredths of the length of the hindbody. It is recurved, with a median concavity facing dorsally. The Mehlis' gland complex and vitelline reservoir are inter-testicular. The vitellaria in the forebody stop short about midway between the pharynx and the acetabulum on the dorsal side. On the ventral side the follicles do not reach the anterior rim of the body but are on a level with the pharynx. In the hindbody the follicles are heavily concentrated in the space anterior to the ovary, whereas posterior to the ovary the follicles occupy a dense ventral strip which extends almost up to the sucker-like genital ring of the genital bursa on the ventral side, and penetrates the genital cone. Copulatory bursa is 0.2 mm. deep and 0.175 mm. in diameter at the level of the genital ring.

The eggs are numerous and measure 0.097 to 0.105 mm. long by 0.064 to 0.076 mm. wide.

RELATIONSHIPS

This species, because of its very small size, and characteristic ovoid shape of the antero-posterior body, differs from the majority of the members of the genus Strigea. With regard to the general morphology, the worm is compared with *S. falconis* Szidat, 1928 ; *S. glandulosa* Dubois, 1937 ; *S. macroconophora* Dubois and Rausch, 1950 and *S. elegans* Chandler and Rausch, 1947.

Amongst other features such as the size of the oral sucker, pharynx and acetabulum the present specimens differ markedly from *S. elegans*, which is a parasite of owls, in possessing a low postero-anterior body ratio, a very much pronounced genital cone and smaller eggs. Both *S. falconis* and *S. macroconophora* are larger forms with correspondingly large testes and ovary, large bursa copulatrix and genital cone and small forebody compared to the hindbody. The Australian species *S. glandulosa* from *Haliastur sphenurus*, is close to the present species in regard to the small body size only. The present species differs from it in the small ratio of the hindbody to the forebody ; the larger oral sucker, pharynx, ventral sucker and eggs ; and in the smaller adhesive gland, ovary and testes.

The species is designated as *Strigea cuncumae* n.sp.

Host : *Cuncuma vocifer vocifer.*

Locality : N. Rhodesia.

Location : Small Intestine.

Type : Department of Parasitology, London School of Hygiene and Tropical Medicine.

STRIGEA RHODESIENSIS N.SP.

Five specimens of this new species were obtained from the rectum of the white-necked vulture, *Pseudogyps, africanus*, in Northern Rhodesia.

The body which lacks the pronounced curvature so characteristic of almost all members of the genus *Strigea*, measures 6 to 8.6 mm. in total length minus the single conspicuous ventral lobe of the holdfast organ, which projects to about a length of 3 mm. from the

anterior rim of the forebody and is almost as long as the forebody, which carries a heavy concentration of vitelline glands. The width of the body across bursa copulatrix is about 1.25 mm. The forebody is 2.8 to 3.86 mm. long by 2.4 to 3.04 mm. wide. The hindbody is 3.2 to 4.75 mm. long by 2.9 to 3.58 mm. wide. It is separated from the forebody by a constriction and the ratio of the forebody to hindbody is between 1.12 to 1.25.

The oral sucker measures 0.29 to 0.36 mm. long by 0.22 to 0.285 mm. wide. The pharynx is almost spherical, 0.18 mm. long by 0.14 to 0.18 mm. wide. Both the oral sucker and pharynx were difficult to locate in *in toto* mounts in four out of the five specimens, due to the projecting lobes of the holdfast organ and the extremely heavy concentration of vitelline follicles in these lobes and in the forebody. In the single specimen in which it was observed both structures were found to project from the dorsal anterior rim of the forebody. Both the ventral sucker, which measures 0.285 mm. long by 0.25 mm. wide and the adhesive gland, 0.42 mm. long by 0.45 mm. wide, lie at the bottom of the cup formed by the forebody. These two structures have been studied in serial sections only, since they were invisible in *in toto* mounts on account of the thickness of the forebody and the massing of vitellaria in this part. The distance between oral and ventral suckers in longitudinal sections varies from about 0.6 to 0.75 mm. A prepharynx is absent. The oesophagus is short, about 0.24 mm. long. Intestinal caeca have a diameter of 0.1 to 0.17 mm. in the first segment and run towards the hind end of the body, ending a short distance from the posterior extremity, lateral to the bursa copulatrix. As seen in sections, the caecal walls appear to be ciliated internally. Large longitudinal muscle processes arising in the anterior segment, mainly near the base of the cup formed by the walls of the first segment, penetrate the hindbody and extend furthermost up to the genital cone.

Abbreviations used in Figs. 7–11.

AG = Adhesive gland ; **B** = Bursa ; **CVD** = Common vitelline duct ; **DE** = Ductus ejaculatorius ; **E** = Egg ; **GC** = Genital cone ; **I** = Intestinal caecum ; **LHF** = Lobes of holdfast organ ; **LC** = Laurer's canal ; **MG** = Mehlis' gland ; **M** = Longitudinal muscle fibres ; **O** = Ovary ; **OES** = Oesophagus ; **OD** = Oviduct ; **OLC** = Opening of Laurer's canal ; **OS** = Oral sucker ; **OT** = Ootype ; **PH** = Pharynx ; **RM** = Ring muscle ; **SA** = Anterior segment ; **SP** = Posterior segment ; **SV** = Seminal vesicle ; **TA** = Anterior testis ; **TP** = Posterior testis ; **U** = Uterus ; **UE** = Egg in uterus ; **V** = Vitellaria ; **VD** = Vas deferens ; **VF** = Vitelline follicle ; **VS** = Ventral sucker.

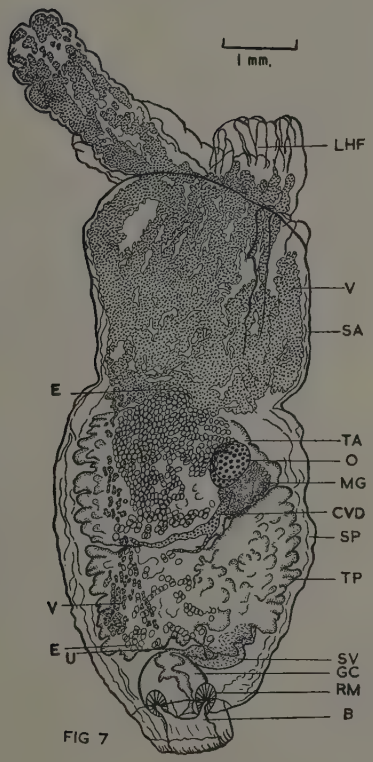


FIG 7

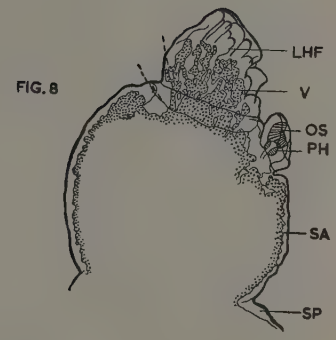


FIG. 8

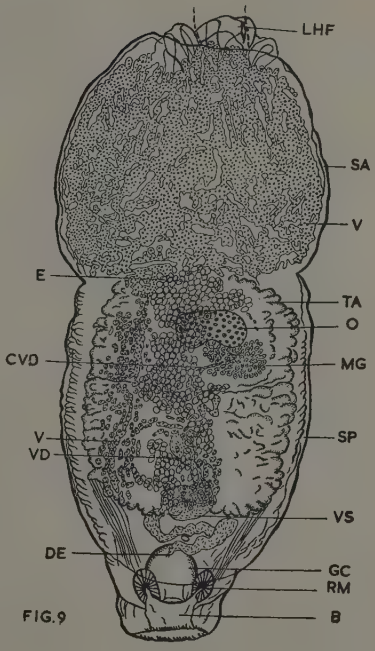


FIG. 9

Strigea rhodesiensis n.sp.

Fig. 7.—Lateral view of entire worm. Fig. 8.—Lateral view of anterior body, showing position of oral sucker and pharynx. (Large ventral lobe not shown.) Fig. 9.—Ventral view of body. (Large ventral lobe not shown.)

The ovary, 0.525 to 0.6 mm. long by 0.465 to 0.54 mm. wide, lies between the 15th to 26th hundredths of the length of the hindbody and is arched dorso-anteriorly and concave ventro-posteriorly in lateral view. When viewed dorso-ventrally the posterior border of the ovary has an almost median indentation. A short oviduct arises from the posterior border of the ovary, lateral to the indentation to enter the ootype, which is situated dorsally between the two testes and is surrounded by numerous Mehlis' gland cells. The Laurer's canal arising from the oviduct opens dorsally. The uterus emerges from the ootype somewhat from its posterior border and dilates after proceeding for a very short distance. It then bends in an anterior direction making a few convolutions to proceed anterior to the ovary and spiralling round many times largely in a horizontal plane reaches in this manner very close to the antero-posterior body division. The uterus, which is full of eggs, measuring 0.105 to 0.117 mm. long by 0.068 to 0.075 mm. wide, enters a spacious genital cone, 0.675 to 0.825 mm. long by 0.68 to 0.95 mm. wide. The large bursa copulatrix has a terminal pore and an average depth of 0.75 mm.

The vitelline glands are accumulated mainly in front of the anterior testis. As stated before the follicles are somewhat heavily concentrated in the forebody, so that a dark-greyish appearance characterises this part of the body in fixed unstained specimens, in contrast to the much lighter colour of the hindbody. The vitellaria penetrate the lobes of the holdfast organ, in front of the anterior rim of the body, and occupy the entire surface of the single large ventral lobe. The glands in the posterior body are comparatively thinly distributed, and are gathered together in two ventral median sheets. Sparcely scattered vitelline material is also found between these two sheets. Two vitelline ducts commence almost equatorially in the hindbody and proceed more or less in an antero-dorsal direction to enter a large yolk reservoir, from which arises a common yolk duct which enters the ootype.

The two testes in the hindbody are very coarsely lobed and tandem, occupying most of the posterior body. The anterior testis measures 1.2 to 1.5 mm. long by 2.8 mm. wide ; the posterior one 1.8 to 2 mm. long by 2.78 mm. wide. The large vesicula seminalis is very much coiled on the postero-dorsal side of the posterior testis. The ejaculatory duct enters the uterus in the midst of the genital cone. A paraprostate is absent.

RELATIONSHIPS

This species differs from any member of the genus *Strigea* hitherto described in lacking a pronounced body curvature, in the concentration of vitelline follicles in a single large ventral lobe of the holdfast organ, which is almost as long as the forebody, in the large very

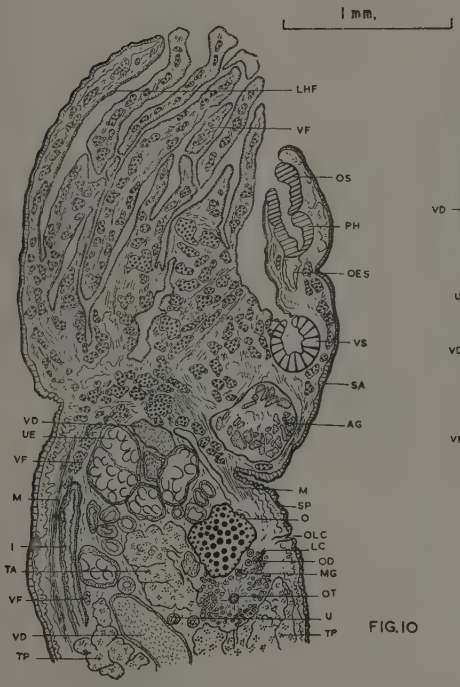


FIG. 10

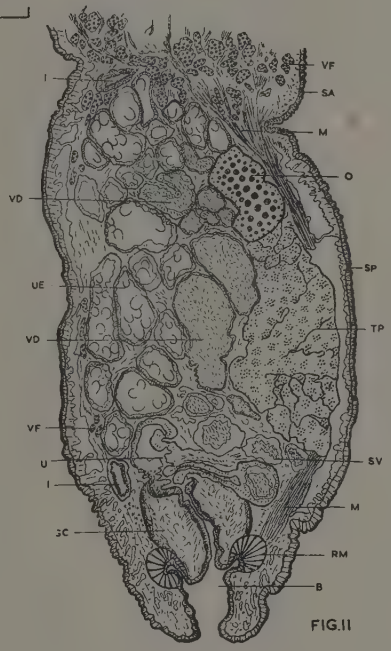


FIG. 11

Strigea rhodesiensis n.sp.

Fig. 10.—Longitudinal section of Anterior segment and anterior half of posterior segment. Fig. 11.—Longitudinal section of posterior segment.

coarsely lobed testes which almost fill the entire hindbody, in the relatively great concentration of vitelline glands in the forebody and in the nature of the uterus. Further, the location of this species is the rectum of the host. It is thus separated from other members of the genus *Strigea*, whose normal habitat is the duodenum and intestine.

The present species agrees with the cosmopolitan species *Strigea falconis* in the body length only. It is far removed from *S. falconis* in the following combination of characters : the relatively large forebody with greater concentration of vitelline follicles ; ratio of the posterior to anterior body ; position of the ovary ; nature of the holdfast organ ; size, location and nature of testes ; size of the eggs ; bursa copulatrix and genital cone. These trematodes are therefore diagnosed as a new species which is designated *Strigea rhodesiensis* n.sp.

Host : *Pseudogyps africanus.*

Locality : Mazabuka, Northern Rhodesia.

Location : Rectum.

Types : Department of Parasitology, London School of Hygiene and Tropical Medicine.

Summary

Four new species of Strigeid Trematodes are described from Central African Birds of Prey. They are : *Strigea neotidis* from *Neotis cafra denhami*, *Strigea lilensis* and *Strigea rhodesiensis* from *Pseudogyps africanus* and *Strigea cuncumae* from *Cuncuma vocifer vocifer*. The relationships of these new forms to other species in the genus *Strigea* are discussed.

Acknowledgments

The writer is deeply indebted to Professor J. J. C. Buckley under whose guidance this work was done. I should like to express my thanks to Dr. P. L. LeRoux for his advice, assistance and material for this paper. I am also thankful to Mr. S. Prudhoe, British Museum (Natural History) for the examination of material and literature.

References

Chandler, A. C. and Rausch, R., 1947.—"A study of Strigeids from Owls in North Central United States." *Trans. Amer. micr. Soc.* **66,** 283–292. (W.L. 21400.)

Dubinin, V. D., 1938.—"Changements de la parasitofaune de *Plegadis falcinellus* L. provoqués par l'age et la migration de l'hote." (In Russian.) *Nauch. Trud. Goszapovedn.* **2,** 114–212. (W.L. 14946c.)

DUBOIS, G., 1938.—Monographie des Strigeida (Trematoda). *Mem. Soc. neuchâtel Sci. nat.*, **6,** 535 pp. (W.L. 13318a.)

———1944.—"A propos de la specificité parasitaire des Strigeida." *Bull. Soc. neuchâtel Sci. nat.*, **69,** 1–103. (W.L. 5299.)

———1953.—Systematique des Strigeida, complement de la Monographie. *Mem. Soc. neuchâtel Sci. nat.*, **8,** 141 pp. (W.L. 13318a.)

DUBOIS, G. and RAUSCH, R., 1948.—"Seconde contribution à l'étude des Strigeides (Trematoda) Nord-Americains." *Bull. Soc. neuchâtel Sci. nat.*, **71,** 29–61. (W.L. 5299.)

———1950.—Troisième contribution à l'étude des Strigeides (Trematoda) Nord-Americains. *Bull. Soc. neuchâtel Sci. nat.*, **73,** 19–51. (W.L. 5299.)

———1950.—"A contribution to the study of North American Strigeids (Trematoda)." *Amer. Midl. Nat.*, **43,** 1–31. (W.L. 640.)

SZIDAT, L., 1928.—"Zur revision der Trematodengattung *Strigea* Abildgaard." *Zbl. Bakt.*, **105,** 204–215. (W.L. 23684.)

———1929.—"Beitrage zur Kenntnis der Gattung *Strigea* (Abildg.) 11. Spezieller Teil : Revision der Gattung *Strigea* nebst Beschreibung einer Anzahl neuer Gattungen und Arten." *Zeit. Parasitenk.*, **1,** 688–764. (W.L. 23536b.)

VERMA, S. C., 1936.—"Notes on the Trematode Parasites of Birds. Part 1." *Allahabad Univ. Stud.*, **12,** 147–188. (W.L. 393c.)

VIDYARTHI, R. D., 1937.—"New Strigeids (Trematoda) from Indian Birds." *Proc. nat. Acad. Sci. India*, **7,** 193–201. (W.L. 16820a.)

YAMAGUTI, S., 1935.—"Studies on the Helminth Fauna of Japan. Part 5. Trematodes of Birds, III." *Jap. J. Zool.*, **6,** 159–182. (W.L. 10881w.)

———1939.—"Studies on the Helminth Fauna of Japan. Part 25. Trematodes of Birds IV." *ibidem*, **8,** 129–210.

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A New Trematode, *Reesella doviensis* gen. et sp. nov., from the Oystercatcher, *Haematopus ostralegus occidentalis*, in Wales

By D. F. Mettrick, B.Sc.

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

In December 1954 Mr. P. W. Davies and myself examined an Oystercatcher, *Haematopus ostralegus occidentalis*, from the Dovey Estuary in Cardiganshire, Mid-Wales. In the mid-intestinal region of the gut we found between fifteen and twenty small trematodes but unfortunately only four of them now remain. These were stained in Celestine blue, but I later demounted two of the specimens and restained in aceto-carmine. During this operation the oesophagus and excretory system were clearly visible although not so in the cleared preparations. They appear to represent a new genus for which I propose the name *Reesella* in honour of Dr. Gwendolen Rees for her contributions to Helminthology.

Psilostomatidae Odhner, 1911, emend. Nicoll, 1935

Reesella n.g.

Generic diagnosis: Psilostomatidae; The body is elongate, flattened and small. The cuticle is spinous anteriorly. The oral sucker is sub-terminal and considerably larger than the ventral sucker, and the pharynx is large and muscular. The intestinal caeca are thick and nearly reach the posterior extremity of the body. The genital pore opens in the mid-line or slightly to one side of it, at the level of the anterior border of the ventral sucker. The cirrus-sac is well developed and curves round the ventral sucker, and may be on the left or right side of the sucker. The cirrus is unarmed. The ovary is large and to the side of the cirrus-sac. The testes are posterior in position, in tandem or slightly oblique to each other, and lying mid-way between the ventral sucker and the posterior extremity of

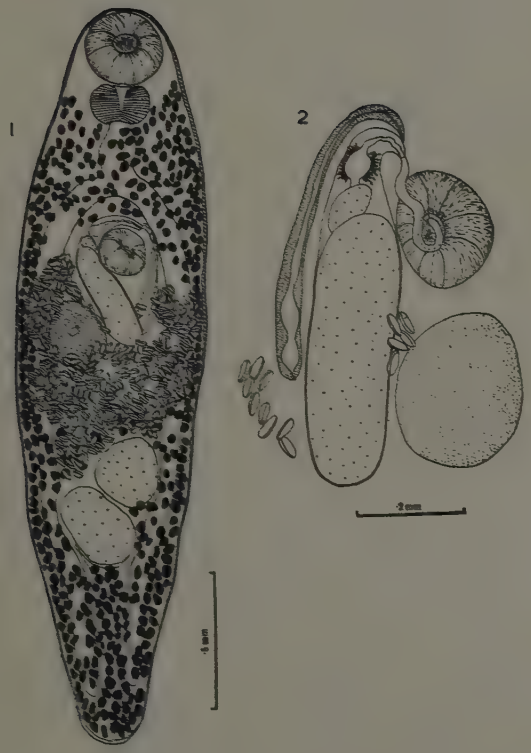
the body. The vitellaria are composed of small follicles extending from the pharyngeal region to the posterior extremity of the body. The excretory system is Y shaped with a short median stem.

Habitat : Intestine of birds.

Genotype : *Reesella doviensis* n.sp.

Reesella doviensis n.sp.

Description : The body is elongated and cylindrical, tapering posteriorly, 2.6 to 4.1 mm. long and 0.62 to 0.8 mm. wide. The cuticle is spinous anteriorly. The oral sucker is sub-terminal, ventral and globular, 0.24 to 0.32 mm. by 0.24 to 0.32 mm. There is a small pre-pharynx. The pharynx is large and oval, 0.14 to 0.17 mm. by 0.15 to 0.2 mm. A short thick oesophagus, 0.15 to 0.16 mm. long by 0.08 to 0.09 mm. wide leads to the bifurcation of the gut. The two intestinal caeca are large and nearly reach the posterior extremity of the body. The ventral sucker is appreciably smaller than the oral, being 0.15 to 0.2 mm. by 0.16 to 0.23 mm. and lying at about one third of the body length. The testes are posterior in position in tandem or slightly obliquely to each other, and lying mid-way between the ventral sucker and the posterior extremity of the body. They are rather variable in shape but are usually regular in outline, the anterior being 0.23 to 0.24 mm. by 0.24 to 0.29 mm. and the posterior being 0.2 to 0.26 mm. by 0.27 to 0.32 mm. The ovary is nearly globular, 0.2 to 0.26 mm. by 0.23 to 0.26 mm., and lies in front of the testes and to the side of the large cirrus-sac. The cirrus-sac is 0.53 to 0.75 mm. long and 0.11 to 0.15 mm. wide, and curves round the ventral sucker to open in the mid-line or slightly to the left of it, and approximately level with the anterior border of the ventral sucker. In the specimens examined the vesicula seminalis is constricted into two portions, is extremely well developed, and occupies up to two thirds of the cirrus-sac. There is also a pars prostatica and a long un-armed extrusible cirrus. The metraterm is well developed and may lie on the same side as or the opposite side to the cirrus-sac, which itself may be on the left or right of the ventral sucker. Mehlis's gland is large and lies posterior to the ovary. The vitellaria are very well developed, composed of numerous small follicles, and extend from the level of the posterior border of the pharynx to the posterior extremity of the body. The follicles meet in the mid-line between the oral and ventral suckers, in front of the anterior testis, and from behind the posterior testis to the posterior extremity of the body. The eggs are large 44 to $48\mu \times 20\mu$, and



Reesella doviensis gen. et sp. nov.

Fig. 1.—Ventral view of whole worm.

Fig. 2.—Metraterm, cirrus-sac, ventral sucker and ovary.

numerous. The main ducts of the excretory system are Y-shaped, the thick unpaired stem branching just behind the posterior testis and opening at the posterior end of the body.

Host : *Haematopus ostralegus occidentalis* Neumann

Location : Intestine.

Locality : Dovey Estuary, Cardiganshire.

Co-types : To be deposited in the collection of the British Museum (Natural History).

Discussion

This new form represents a genus which appears to be an intermediate type between other genera whose phylogeny has not yet been clearly stated. It has close affinities with two particular genera, i.e. *Ribeiroia* Travassos, 1939 and *Psilostomum* Looss, 1899.

Travassos' original definition of *Ribeiroia* was based on only one species, *R. insignis* Travassos 1939. Price (1942) transferred his species *Psilostomum ondatrae* Price, 1931 to this genus, and suggested that it probably was synonymous with *R. insignis*. Dollfus (1950), when describing a new species *Ribeiroia congolensis*, slightly modified Travassos' definition in order to admit this third species to the genus. The modifications made were that the vitellaria meet or nearly meet in the mid-line behind the posterior testis, that the entire borders of the testes may be more or less lobed, and that the genital pore is median or a little to the left of the mid-line. (It appears from the text that Dollfus has overlooked Price's paper.)

Travassos when discussing the affinities of *Ribeiroia* to other genera, said that he considered it an intermediate type between *Trifolium* Travassos, 1922, and *Cathaemasia* Looss, 1899, and placed it in the sub-family *Omphalometrinae* of the family *Echinostomatidae*. He included his new genus with *Omphalometra* Looss, 1899, *Cathaemasia* Looss, 1899, *Trifolium* Travassos, 1922, *Pulchrosoma* Travassos, 1916, and *Pulchrosomoides* Freitas & Lent, 1937.

Dollfus (1950), however, erected a new sub-family *Cathaemasiinae* in which he provisionally placed the three genera *Cathaemasia* (including *Pulchrosoma*), *Ribeiroia*, and *Cathaemasioides*. Even after removing these three genera from the *Omphalometrinae* Dollfus

considers it a heterogenous group because of its affinities as recognised by Odhner, 1911. Moreover Dollfus does not agree with Travassos that the *Omphalometrinae* is a sub-family of the *Echinostomatidae*.

When Price (1942) reviewed the genus *Psilostomum* Looss, 1899, he stated that there were then eight species in the genus, five of which he regarded as valid, including his new species *P. marilae* from the lesser scaup duck, *Marila affinis*.

Skrjabin (1947), however, listed nine valid species including *Psilostomum lineatum* (Linton, 1928) from the herring gull, which Odhner (1928) showed to be identical with *Podocotyle olssoni* a common parasite of fishes; *Psilostomum plicitum* (Linton, 1928) also from the herring gull, which Stunkard (1931) declared was identical with *Bianium concavum* (Stunkard, 1930) another fish parasite and whose correct name is now *Bianium plicitum* (Linton, 1928) Stunkard, 1930; *Psilostomum ondatrae* (Price, 1931) which Price (1942) had correctly transferred to the genus *Ribeiroia*; and *Psilostomum arvicolae* Schulz and Dobrova, 1933, which was described from a single specimen from which the anterior end was missing, and is probably an Echinostome.

Cercariae obtained from *Stagnicola reflexa* were regarded by Feldman (1941) as identical with *Cercaria reflexae* Cort, 1914, and the adult placed in the genus *Psilostomum*. This was questioned by Price (1942), and Beaver (1943) transferred *Psilostomum reflexae* to his new genus *Protechinostoma* under the name *P. mucronisertulatum* declaring that there was no justification for stating that its cercaria was identical with *C. reflexae* Cort, 1914.

As *Psilostomum brevicolle* (Creplin, 1829) is the type species of the genus the validity of assigning three other species to it is questionable. They are *P. progeneticum* Wisniewski, 1933, *P. marilae* Price, 1942, which Price considered as closely resembling *P. progeneticum* and *P. varium* Linton, 1928. *P. marilae* appears to be more closely related to forms in the genus *Psilotrema* Odhner, 1913, and I suggest that it be removed to that genus. The other two species also have characters considerably at variance with those of the genotype, and when further studied they may have to be placed in one or two new genera.

Thus the two genera with which the above described form has affinities are at present in different families. The placing of *Ribeiroia* in the family *Cathaemasiidae* is questionable, as it has close

affinities with the genera in the family *Psilostomatidae* in which I have provisionally placed this new genus.

Summary

1 A new trematode *Rossella dorrensis* n.g., n.sp., from the Oystercatcher, *Haematopus ostralegus occidentalis*, is described.

2 The validity of including *P. progeneoides*, *P. marilae* and *P. varium* in the genus *Psilostomum* is questioned and it is suggested that *P. marilae* be removed to the genus *Psilorchis* Odhner 1913.

3 The placing of the genus *Joyeuxia* in the family *Lecithodendriidae* is also questioned, and it is suggested that it has closer affinities with the genera in the family *Psilostomatidae*.

Acknowledgments

I am indebted to Prof. J. J. C. Buckley under whose supervision this work was carried out, and to Mr. S. Prudhoe of the British Museum for constructive criticism and suggestions. This work was carried out during the tenure of a grant from the Agricultural Research Council.

References

Beaver, P. C. 1943. "Studies on *Prosthogonimus macrorchis* n.sp. and *Prosthogonimus ovatus* Rudolphi 1803." *J. Parasit.*, **29**, 65–70. (W.L. 11428)

Dollfus, R. P. 1950. "Trematodes recoltes au Congo Belge par le Professeur Paul Bouvier." *Ann. Mus. Congo Belge*, C. Serie 3, 185 pp. W.L. 881)

Eguchi, K. 1941. "Studies on the morphology and biology of a psilostome fluke." *Parasit.*, **33**, 525–535. (W.L. 11428).

Linton, E. 1928. "Notes on trematode parasites of birds." *Proc. U.S. nat. Mus.*, **73**, 1–30. (W.L. 11892).

Odhner, T. 1928. "Weitere Trematoden mit Anus." *Ark. Zool.* Stockholm **20**, 1–6.

Price, E. W. 1943. "A new trematode of the family *Psilostomidae* from the brown pelican, *Pelecanus occidentalis*." *Proc. helm. Soc. Wash.*, **9**, 30–31. (W.L. 9276)

Skrjabin, K. I. 1948. "Trematoda of Animals and Man." Principles o Trematodology. Vol. 2. Moscow-Leningrad. pp. 515.

Sudarikov, V. E. 1959. "Further observations on the occurrence of anal openings in digenetic trematodes." *Z. Parasitenk.*, **3**, 715–725. (W.L. 14000).

Travassos, L. 1931. "Um novo trematoden parasito da garcas *Ribeiroia progenes* n.g. n.sp." *Bol. biol.*, **4**, 301–304. (W.L. 3061).

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*On Two New Species of *Lytocestus* from Burma and the Sudan Respectively

By Jean A. Lynsdale, B.Sc., Ph.D.

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

The sub-family Lytocestinae was created by Hunter (1927) to accommodate five genera—*Lytocestus* Cohn, 1908 (type genus), *Balanotaenia* Johnston, 1924, *Monobothrioides* Fuhrmann and Baer, 1925, *Djombangie* Bovien, 1926, and *Lytocestoides* Baylis, 1928. He based the characteristics of the sub-family on the broad characters of the type genus, e.g. "Sub-family Lytocestinae Hunter, 1927. Sub-family diagnosis : Caryophyllaeidae with sexual apertures and ovary situated in the last quarter of the body length. The inner longitudinal muscles lie *entirely internal* to the vitellaria which are annularly arranged about the muscles in the cortical parenchyma. Uterine glands are present. Type genus *Lytocestus* Cohn, 1908." All the foregoing characteristics are present in *Lytocestus*. Subsequently, two more genera, *Khawia* Hsu, 1935, and *Stocksia* Woodland, 1937 have been added, making a total of seven genera.

According to Woodland (1926) the genus *Lytocestus* includes the following species : *L. adhaerens* Cohn, 1908 (type species), *L. filiformis* (Woodland, 1923), *L. chalmersius* (Woodland, 1924), *L. cunningtoni* (Fuhrmann and Baer, 1925), *L. indicus* (Moghe, 1925), and *Balanotaenia bancrofti* Johnston, 1924.

Hunter (1927) sifted through the genus, as he felt that it contained many questionable forms and retained only three species : *L. adhaerens* Cohn, 1908 (type species), *L. filiformis* (Woodland, 1923) and *L. indicus* (Moghe, 1925). He recognised the validity of *Monobothrioides cunningtoni* Fuhrmann and Baer, 1925 and found that *L. chalmersius* (Woodland, 1924) fell within that genus. He

* Part of a thesis approved by the University of London for the award of the Ph.D. degree.

also recognised the validity of Johnston's (1924) *Balanotaenia bancrofti* and removed it from the genus *Lytocestus*. Therefore, there remain the three species enumerated above and to them the writer adds two new species, the descriptions of which now follow. The writer believes that this is the first record of the occurrence of *Lytocestus* in Burma and the second in the Sudan. The new species from the Sudan is recorded from a new host, a Cyprinid fish ; other species of *Lytocestus* occur in Mormyrid and Siluroid fishes.

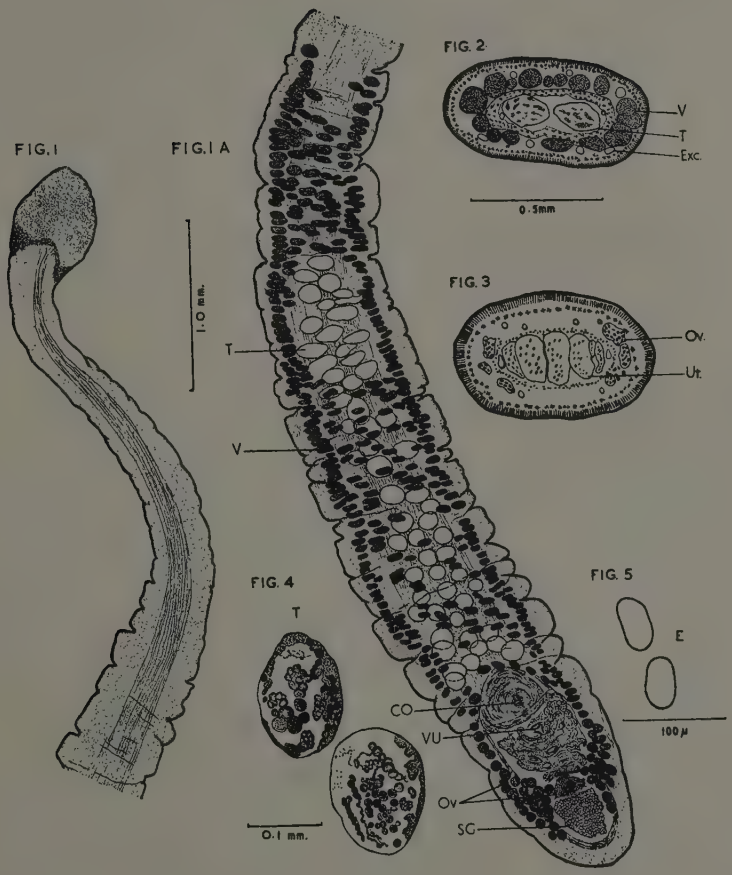
Lytocestus birmanicus n.sp.

These worms were collected from the intestine of *Clarias batrachus* in Rangoon, Burma and consisted of roughly twenty-five specimens in varying degrees of contraction. Twelve worms were measured, and the lengths were found to vary from 5–6 mm. in the contracted specimens and 10–12 mm. in the extended ones. The breadth of the latter was 0.9 mm. in the body region, while that of the former was considerably more.

The body of an extended specimen is elongated and flattened without any trace of internal segmentation, though wrinkling is extensive and in some parts the grooves extend right across the body giving it a segmented appearance (Fig. 1).

The head is lanceolate and measures 0.8 mm. long by 0.5 mm. broad. It is smooth, and narrows to form the neck which widens gradually into the anterior part of the body which is devoid of any organs. This is followed by the broader main region of the body which tapers towards the rounded posterior end. The narrow head region as compared to the body region is one of the less important characteristics of all species of *Lytocestus*, but it is nevertheless, a constant one.

The longitudinal muscle system is seen in transverse sections (Figs. 2 and 3) to consist of two well-marked layers : (1) the outer longitudinal muscle layer, or cortical layer, consisting of small scattered bundles of muscle fibres lying below the nuclear layer of the subcuticula and external to the vitellaria ; (2) the inner longitudinal muscle layer, or epimedullary layer, composed of a broad band of large bundles of muscle fibres, lying internal to the vitellaria just outside the medullary parenchyma, and enclosing the testes.



Lytocestus birmanicus n.sp.

Fig. 1, Fig. 1A.—Entire worm. Fig. 2.—Tranverse section, testicular region. Fig. 3.—Transverse section, uterine region. Fig. 4.—Testes under high magnification. Fig. 5.—Eggs.

CO = Cirrus opening; **E** = Eggs; **Exc.** = Excretory canal; **Ov** = Ovary; **SG** = Shell glands; **T** = Testis; **Ut** = Uterus; **V** = Vitellaria; **VU** = Vagino-uterine opening.

The excretory system in the testicular and uterine regions consists of two medullary vessels, one on each side, external to the testes or uterus, and other, narrower cortical vessels situated between the epimedullary and cortical muscle layers.

TABLE I

	Lytocestus indicus Moghe, 1931	*Lytocestus birmanicus* n.sp.
Length	15–29 mm.	10–12 mm.
Breadth	1.82–2.73 mm.	0.9 mm.
Scolex	L 3 mm. × B 1.2 mm.	L 0.8 mm. × B 0.5 mm.
Vitellaria	88–112μ × 77–88μ	100–120μ × 40–60μ
Testes	119μ × 95μ	150–180μ × 100–130μ
Eggs	80μ × 40μ	50μ × 30μ
Distance between genital pores	220–270μ	180μ

From the apex of the scolex to the most anterior vitellaria the distance is about 4 mm., i.e., about one-third of the length of the body. The vitelline glands are situated in the cortical parenchyma (Fig. 2) and are arranged in an annular manner inside the outer longitudinal muscle layer and outside the inner longitudinal muscle layer. They extend for nearly two-thirds of the length of the body, as far as the vagino-uterine opening but not beyond it. They are elongated transversely and measure 100–120μ broad by 40–60μ long. In the *in toto* mount (Fig. 1A) they appear to be concentrated in two lateral bands on either side of the body but some are also scattered in the median field, but these do not extend posterior to the region of the cirrus pouch.

The numerous testes occur a short distance behind the anterior vitellaria, lie internal to them in the medullary parenchyma (Fig. 2) and are limited to the median field. They are oval or spherical in shape, and measure 150–180μ long by 100–130μ broad.

The ovary lies at the posterior end of the body, and consists of numerous follicles arranged in two wing-like masses on either side, united by a median isthmus. The ovarian follicles are cortical (Fig. 3) and the isthmus is medullary ; the latter also contains ova. In all the worms examined by the writer, the ovarian follicles always extended to the posterior level of the shell gland (Fig. 1A). The shell gland is posterior to the ovary and consists of a more or less rounded mass with its anterior face flattened.

The uterus consists of a number of loose coils, the most anterior loops reaching the space between the two genital openings. Eggs in the uterus measure 50μ long by 30μ broad.

The genital openings are at the beginning of the last one-seventh of the body, and consist of the opening of the cirrus sac, and the common vagino-uterine opening; the distance between the two openings is 180μ.

In general anatomy, the writer's specimens approximate most closely to Moghe's (1931) *Lytocestus indicus*, but differ from it in several respects. In Table I the main differences between the two worms with regard to measurements, are indicated.

From this Table it is apparent that there is a marked discrepancy between the lengths and breadths of the two worms, the minimum length of *L. indicus* being greater than the maximum length of *L. birmanicus* and the minimum breadth of the former being twice the maximum breadth of the latter. Similarly the scolex of the former is much larger than that of the latter. Little or no emphasis is placed in the literature on the macroscopic measurements of the species of *Lytocestus*, but the comparisons just drawn show that *L. birmanicus* is a decidedly smaller form than *L. indicus*. With reference to internal structure, the vitellaria and testes of *L. indicus* are slightly smaller than those of *L. birmanicus*.

In addition to the above differences, Moghe (1931) describes the scolices of some of his worms as being marked with longitudinal furrows similar to those of *Monobothrioides cunningtoni* Fuhrmann and Baer, 1925 and *Monobothrioides chalmersius* (Woodland, 1924), but these are completely absent in the writer's specimens.

The most marked and constant difference however, lies in the position of the ovarian follicles with reference to the shell gland. Moghe's (1931) description and figure of *Lytocestus indicus* show clearly that the ovarian follicles never reach the anterior level of the shell gland, whereas in the writer's specimens the ovarian follicles, without exception, extend to the posterior level of the shell gland.

The writer therefore feels that these characteristics may be considered sufficient for regarding these worms as a new species and accordingly they are named *Lytocestus birmanicus* n.sp.

Lytocestus alestesi n.sp.

The material of the above species consists of a slide of a single worm from *Alestes nurse*, Sudan. It is unfortunately in four pieces and owing to its broken condition, there is the possibility of inaccuracy in the length, otherwise all other measurements may be taken as unaffected by the fragmentation. The writer is indebted to Professor Sandon, University College of Khartoum, for supplying this material.

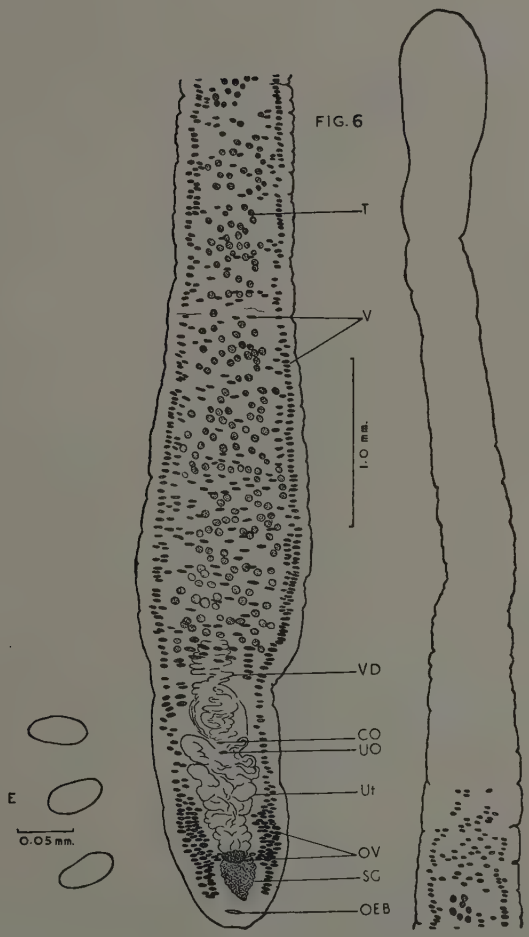
The worm has a length of about 10.5 mm., and a maximum breadth of a little more than 1 mm. The body is elongated and flattened; there is no trace of external or internal segmentation and wrinkling is very slight.

The head is flattened and ribbon-like with a rounded apex. From the apex of the head to the most anterior vitellaria, the distance is a little more than 4 mm., i.e. about two-fifths of the length of the body. The vitelline glands extend from a short distance behind the most anterior testes up to the anterior tips of the horns of the ovary, but not beyond them. They are elongated transversely, and measure 40–50μ broad by 20μ long. They lie external to the testes, and are distributed laterally as well as in the median field, but the latter do not extend beyond the cirrus pouch.

The testes are numerous, and are limited to the median field; their extent is indicated in Fig. 6. They are more or less spherical in shape and measure from 50–70μ long by 40–60μ broad.

The ovary lies at the extreme posterior end of the body, and consists of a distinctly bi-lobed mass united by a median isthmus; behind it is the shell gland, a rounded body with its anterior face flattened. The ovarian follicles extend to the posterior level of the shell gland. The post-ovarian region is negligible, the excretory pore opens here and is sub-terminal in position.

The uterus is very short and lies in loose coils, and opens together with the vagina through the vagino-uterine pore. The two genital openings are distinctly separate, there being a distance of 40μ between the posterior border of the male opening, and the anterior border of the female opening. Eggs in the uterus measure 40–50μ long by 25–30μ broad.



Lytocestus alestesi n.sp.

Fig. 6.—Entire worm.

CO = Cirrus opening; **E** = Eggs; **OEB** = Opening of excretory bladder; **Ov** = Ovary; **SG** = Shell glands; **T** = Testis; **UO** = Vagino-uterine opening; **Ut** = Uterus; **V** = Vitellaria; **VD** = Vas deferens.

No sections are possible in the circumstances, therefore details of musculature cannot be determined. However, in general arrangement of genitalia, the worm closely resembles the writer's *Lytocestus birmanicus* n.sp., described in the preceding pages. This resemblance is especially marked in the extent of the ovarian follicles which reach the posterior level of the shell gland, but not beyond it. This characteristic is a very constant one in all the worms examined by the writer, and placed in her new species. On the other hand, there are many disparities between *Lytocestus birmanicus* and *Lytocestus alestesi* in the size of various structures which are of significance. In Table II are shown the differences between the two forms.

TABLE II

	Lytocestus birmanicus n.sp.	*Lytocestus alestesi* n.sp.
Length	10–12 mm.	10.5 mm.
Breadth	0.9 mm.	1 mm. plus
Testes	L 150–160μ × B 100–130μ	L 50–70μ × B 40–60μ
Vitellaria	L 100–120μ × B 40–60μ	L 40–50μ × B 20μ
Eggs	50 × 30μ	40–50μ × 25–30μ
Distance between genital pores	180μ	40μ
Excretory bladder	Terminal	Sub-terminal

It is apparent from Table II that, apart from similarities in length and breadth, the differences in the other measurements preclude the possibility of identifying one worm with the other.

There are moreover the other very important differences, namely, in their hosts, and particularly in the geographical distribution of these hosts, as the geographical distribution of a cestode must necessarily depend upon that of its host. The host of *Lytocestus birmanicus* is *Clarias batrachus*, a Siluroid fish in Burma, and that of *Lytocestus alestesi* is *Alestes nurse*, a Cyprinid fish in the Sudan. Up to the present time no species of *Lytocestus* has been reported from a Cyprinid host, the adults being confined to Mormyrid and Siluroid fishes only. There is no really satisfactory way to account for the solitary *Lytocestus* in a Cyprinid host, but three possibilities may be considered :—

(1) that mis-labelling, or a mixing of labels may have occurred, though the writer has been assured that, in this instance, the possibility is remote ; (2) that a small Siluroid fish containing the

worm was eaten by a larger Cyprinid fish, and that the worm continued to live in the intestine of its new host; (3) that the intermediate host of the worm, be it a Tubificid worm or an Entomostracan, as is the case in the life cycle of other tapeworms, was eaten by the Cyprinid fish and the larva developed within it into the adult. The second and third conditions would constitute a case of pseudo-parasitism, which according to Meggitt (1934) is the occurrence of a parasite outside its normal host range.

It appears to the writer that the third condition is the most probable.

Until more worms from the Cyprinid host are available for further examination, the writer is of the opinion that this worm must be accepted in the genus *Lytocestus* Cohn, 1908. It obviously does not belong to the genus *Lytocestoides* Baylis, 1928, the only other *Lytocestus* reported from *Alestes*, as the latter possess post-ovarian vitellaria which are completely absent in the writer's worm. As regard species, it differs from the only other Sudan species of *Lytocestus* described by Woodland, i.e. *Lytocestus filiformis* (Woodland, 1923) in the fact that the vitellaria extend as far as the posterior level of the shell gland in the writer's worm, whereas in *L. filiformis* they stop at the anterior level of the ovary. The eggs also differ in size, those of the writer's worm being two-thirds the length of those of *L. filiformis* which are exceptionally long. The host of the two forms also differ, that of the latter being a Mormyrid fish.

Therefore, though the writer is not in favour of creating a new species on the evidence of a single worm, there does not appear to be any alternative, and she accordingly names this worm *Lytocestus alestesi* n.sp.

REFERENCES

COHN, L., 1908.—"Die Anatomie eines neuen Fischcestoden." *Zbl. Bakt.*, **46**, 134–139. (W.L. 23684.)

FUHRMANN, O. and BAER, J. G., 1925.—"Zoological results of the third Tanganyika Expedition conducted by Dr. W. A. Cunnington, 1904–5. Report on the cestoda." *Proc. zool. Soc. Lond.* 79–100. (W.L. 16969b.)

HUNTER, G. W. III., 1927.—Studies on the Caryophyllaeidae of North America. *Illinois biol. Monogr*, **11**, 371–556.

———1927.—"Notes on the Caryophyllaeidae of North America." *J. Parasit.*, **14**, 16–26. (W.L. 11428.)

———1929.—"New Caryophyllaeidae from North America." *J. Parasit.*, **18**, 185–192. (W.L. 11428.)

JOHNSTON, T. H., 1924.—"An Australian caryophyllaeid cestode." *Proc. Linn. Soc. N.S.W.* **49,** 339–347.

MOGHE, M. A., 1925.—"*Caryophyllaeus indicus*, n.sp. (Trematoda) from the catfish (*Clarias batrachus* Bl)." *Parasitology*, **17,** 232–235. (W.L. 16035.)

———1931.—"A supplementary description of *Lytocestus indicus* Moghe (Syn. *Caryophyllaeus indicus* Moghe, 1925. Cestoda)." *ibid.*, **23,** 84–87.

WOODLAND, W. N. F., 1923.—"On some remarkable new forms of Caryophyllaeidae from the Anglo-Egyptian Sudan and a revision of the families of the Cestodaria." *Quart. J. micr. Sci.*, **67,** 435–472. (W.L. 17510.)

——1924.—"On a new species of the cestodarian genus *Caryophyllaeus* from an Egyptian siluroid." *Proc. zool. Soc. Lond.* 529–532. (W.L. 16969b.)

———1926.—"On the genera and possible affinities of the Caryophyllaeidae. A reply to Drs. O. Fuhrmann and J. G. Baer." *ibid.*, 49–69.

———1937.—"Some cestodes from Sierra Leone. I. On *Wenyonia longicauda* sp.n. and *Proteocephalus bivitellatus* sp.n." *ibid.*, 931-937.

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On a Redescription of *Foleyella candezi* Fraipont, 1882, from Meller's Chamaeleon *(Chamaeleon melleri)*, from Nyasaland

By L. G. Jayewardene, L.M.S., D.T.M.&H., Ph.D.

Medical Research Institute, Colombo, Ceylon.
From the Department of Parasitology, London School of Hygiene and Tropical Medicine.

Two species of nematodes were recovered from the Meller's chamaeleon from Nyasaland, which died at the London Zoo. They comprised ten females and eight males of *Foleyella candezi* from the axilla and peritoneal cavity, and two adult females of *Abbreviata* sp. from the gut. Specific determination was not possible for the latter species, owing to the paucity of the material.

Filariidae (Cobbold, 1864) Claus, 1885.
Foleyella Seurat, 1917
Foleyella candezi Fraipont, 1882.
(Syns. *F. agamae* Rodhain, 1906; *F. pigmentata* Kreis, 1945)

The *females* are thread-like and very slightly flattened dorso-ventrally. They have an average length of 94.8 mm. and range from 73 to 124 mm. The width is more or less uniform, average 0.5 mm. but becomes narrow at 1 mm. from the tip of the tail. The anterior end is bluntly rounded and in some specimens has a slightly bulbous appearance when observed from the lateral aspect. This is due to a slight narrowing at the junction of the oesophagus and intestine.

The tail is more attenuated than the anterior end and it is slightly curved ventrad and marked by a shallow groove running ventrally from the anus to the tip where it is flanked by two minute papillae-like structures.

The shallow stoma is somewhat oval in shape and surrounded by ten minute papillae, six of which form an inner circle and four an outer circle. The mid-dorsal and mid-ventral papillae of the inner circle are more conspicuous than the others.

The oesophagus is separated into an anterior muscular and a posterior glandular part. Demarcation occurs at the level of the nerve ring. The average length of the oesophagus is 0.73 mm. the anterior portion being about one fifth of the posterior.

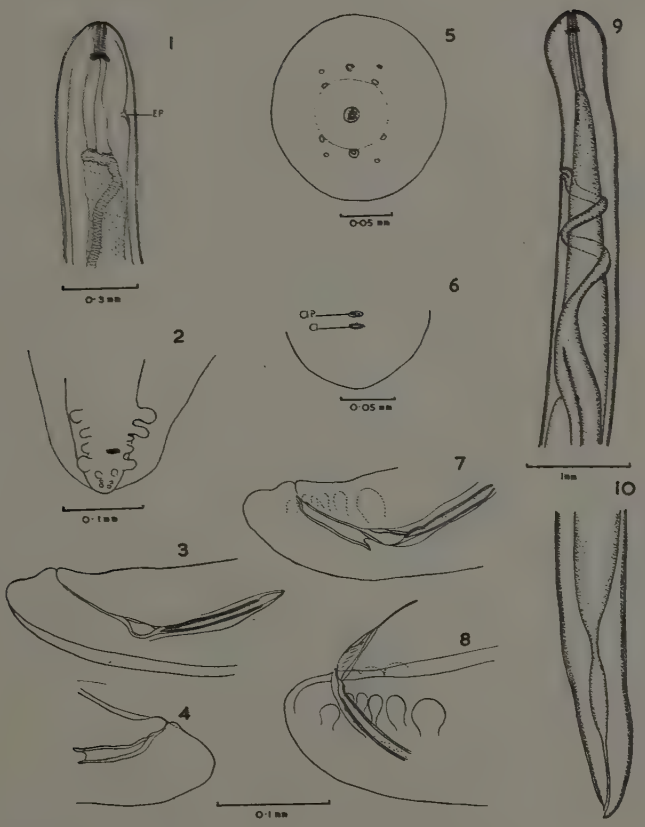
The intestine is a wide thin-walled tube, which narrows suddenly to form the rectum. The average length of the rectum is 2 mm. Chitinisation of the rectum is not very obvious. The anal opening is inconspicuous, subterminal in position and situated 0.1 mm. from the tip of the tail.

The vulva is situated at the junction of the oesophagus and intestine or behind it, its distance from the anterior end of the oesophagus varying from 0.78 to 1.7 mm. The vagina, formed by the union of the two uterine tubes, has an average length of 3.3 mm. It twists spirally round the terminal portion of the intestine and opens ventrally at the slit-like aperture of the vulva. In older specimens, the loops of the vagina displace the oesophagus and intestine, and anteriorly extend as far forward as the nerve ring.

The excretory pore is situated about midway between the anterior end and the vulva. Cervical papillae were not observed.

The *male* is thinner than the female and has an average length of 28 mm. The tail is occasionally straight, but often curved or coiled in one or more spirals. The body is of uniform width, 0.2 to 0.3 mm., but begins to narrow at the commencement of the caudal alae, 2.5 mm. from the tip of the tail. The tail is 0.5 mm. long. The cuticle is devoid of striations.

The caudal papillae consist of a ventral and a ventro-lateral group. The ventro-lateral group is asymmetrically disposed, generally consisting of five to six papillae on the left and four on the right. These papillae are large and pedunculated, the anterior pair being the largest. They gradually decrease in size posteriorly, but the most posterior pair, which is post-anal in position, is considerably larger than the adanal pair which immediately precedes it. The ventral group consists of a single preanal papilla, elliptical in shape,



Foleyella candezi

Fig. 1.—Anterior end of male. Fig. 2.—Ventral view of male tail. Figs. 3 and 4.—Lateral views of male tail. Fig. 5.—End-on view of head. Fig. 6.—Cloaca and pre-cloacal papilla of male. Figs. 7 and 8.—Lateral views of male tail. Fig. 9.—Anterior end of female. Fig. 10.—Tail of female. Abbreviations. Cl.—Cloaca. Cl.P.—Pre-cloacal papilla. EP.—Excretory pore.

situated immediately anterior to the anal opening, and three pairs of small sessile post-anal papillae—the most anterior of these, again being the largest.

There is a long left and a short right spicule. The left spicule consists of a handle, about 0.09 mm. long and 0.01 mm. wide, followed by a membranous portion 0.02 mm. wide. This membranous portion is shaped rather like the keel petal of a legume, being open ventro-laterally. Its distal end is prolonged and resembles a fine stillette. This spicule has an average length of 0.18 mm. The right spicule is shorter, broader and boat-shaped. Its proximal end has two projections at each side, the left protruding more anteriorly than the right. The distal end is directed ventrally and to the left. The average total length of this spicule is 0.08 mm. and has a maximum width of a little over 0.02 mm. In at least five specimens examined, this spicule was not seen to extend outside the cloaca, except for its extreme tip.

*Table I

	Male	Female
Length of body	25–37 (7)	73–124 (11)
Maximum width of body	0.2–0.3 (5)	0.5–0.7 (3)
Length of oesophagus	0.52–0.62 (5)	0.70–0.75 (6)
Width of muscular oesophagus	0.04–0.07 (3)	0.06–0.07 (3)
Width of glandular oesophagus	0.1–0.11 (3)	0.1–0.15 (3)
Excretory pore to anterior end	0.35–0.4 (3)	0.46–1.0 (4)
Nerve ring to anterior end	0.10–0.12 (5)	0.14–0.18 (4)
Length of tail	0.05 (5)	0.1 (4)
Length of left spicule	0.16–0.185 (5)	—
Length of right spicule	0.075–0.095 (5)	—
Distance of testicular loop from anterior end	0.46–0.82 (5)	—
Vulva from anterior end	—	0.8–1.7 (4)
Length of vagina	—	3.0–3.7 (3)

* All measurements are in millimetres.

The coils of the testicular loop extend to a varying distance anteriorly, ranging from 0.46 to 0.83 mm. from the anterior end. In older specimens it was seen to displace the oesophagus.

Table I gives the respective measurements of the male and female worms. The figures within ( ) in the table represent the number of specimens measured in each instance.

DISCUSSION

Our material agrees very well with the description and measurements of *Filaria candezi* Fraipont, 1882, *Filaria agamae* Rodhain, 1906, and *Foleyella pigmentata* Kreis, 1945.

*TABLE II

	F. candezi	*F. agamae*	*F. pigmentata*	Present material
Length of male	25	25–40	24	25–37
Length of female	69	84	80–83	73–124
Length oesophagus, male ...	0.576	0.550	0.55–0.56	0.52–0.62
Length oesophagus, female ...	0.720	0.663	0.9–1.0	0.7–0.75
Left spicule	0.175	†0.200	0.186	0.160–0.185
Right spicule	0.050	†0.090	0.065	0.975–0.095

* All measurements in mm. †Estimated from drawing.

The description and illustrations of the spicules in the above group correspond very closely, but there is a difference in the ratio of the length of the short spicule to that of the long spicule. In *Filaria candezi* and *Foleyella pigmentata* the ratio is approximately as 1 : 3, while in *Filaria agamae* and the present material, it is as 1 : 2. In addition *Foleyella pigmentata* Kreis, 1945, is distinguished by the presence of pigment in the cephalic region. Pigment was not seen in any of our specimens.

From the examination of our material, we believe that *Filaria candezi* Fraipont, 1882, *Filaria agamae* Rodhain, 1906, and *Foleyella pigmentata* Kreis, 1945 should be regrouped under the name *Foleyella candezi*.

REFERENCES

BREINL, A., 1911.—"Nematodes observed in North Queensland." *Rep. Aust. Inst. trop. Med.*, 39–48. (W.L. 17930c)

COWPER, S. G., 1945.—"Some observations on a filaria, *Foleyella leiperi* Railliet, 1916 of the North American leopard frog." *Ann. trop. Med. Parasit.*, **39,** 119–124. (W.L. 1063)

———— 1946.—"A further note on the nomenclature and identity of the frog filaria." *Ibidem*, **40,** 171–172.

GEDOELST, L., 1916.—"Notes sur la faune parasitaire du Congo Belge." *Rev. zool. afr.*, (1916–18), **5,** 46–47 (W.L. 19323).

KREIS, H. A., 1938.—"Beiträge zur Kenntnis parasitischer Nematoden VIII Neue parasitische Nematoden aus dem Naturhistorischen Museum Basel." *Zbl. Bakt.*, **142,** 329–352. (W.L. 23684)

———— 1945.—"Beiträge zur Kenntnis parasitischen Nematoden XII Parasitische Nematoden aus den Tropen." *Rev. suisse Zool.*, **52,** 551–596. (W.L. 19288)

LEIPER, R. T., 1908.—"An account of some helminths, contained in Dr. C. M. Wenyon's collection from the Sudan." *Third Rep. Wellcome trop. Res. Lab.*, 187–199 (W.L. 18653).

RAILLIET, A., 1916.—"Sur les Filaires de Batraciens." *Bull. Soc. Pat. exot.*, **9,** 137–140. (W.L. 5310)

RODHAIN, J., 1922.—"Sur une filaire parasitant le tissue conjunctif sous cutane de *Agama colonarum* Dum et Bibr. au Congo Belge." *C. R. Soc. Biol., Paris*, **87,** 807–810. (W.L. 6630)

SEURAT, L. G., 1916.—"Sur deux filaires des reptiles du Nord-Africain." *Ibidem*, **79,** 1131–1136.

———— 1917.—"Filaires des Reptiles et des Batraciens." *Bull. Soc. Hist. nat. Afr. N.*, **8,** 236–242. (W.L. 5131)

WEHR, E. E. and CAUSEY, O. R., 1939.—"Two new nematodes (Filarioidea : Dipetalonematidae) from *Rana sphenocephala*." *Amer. J. Hyg.*, **30,** 65–68. (W.L. 600a)

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On Some Spirurid and Filariid Nematodes of Birds in Pakistan

By M. M. Sarwar, L.V.P., B.V.Sc., Ph.D.
Punjab College of Animal Husbandry, Lahore

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

This paper deals with material collected partly from birds shot at Sheikhupura (Punjab) and partly from birds dying in the Zoological Gardens, Lahore.

Histiocephalidae Skrjabin, 1941
Stellocaronematinae Skrjabin, 1941
Stellocaronema Gil'Bert, 1930
Stellocaronema buckleyi sp. n.

About two dozen worms of this new species were collected from a specimen of the Indian Red-wattled Lapwing, *Lobivanellus indicus indicus*, shot at Sheikhupura. The worms were found lying in sinuous galleries under the horny layer of gizzard.

The worms are long and slender and in both sexes taper gradually towards their anterior extremities. The females are about two to three times as long as the males. The mouth comprises a dorso-ventrally elongated oral opening surrounded by two well developed pseudolabia. The pseudolabial set of papillae comprise one pair of lateral and two pairs of submedian. The cephalic appendages consist of four pairs of tooth-like cuticular projections extending posteriad from the base of the head. The appendages are seen to be star shaped in the *en face* view of the head. The four great submedian papillae of the external ring are not visible from this view. The cuticle of the worms is smooth and finely striated transversely.

Males measure 8 to 9 mm. long and have a width of 0.14 to 0.15 mm. immediately anterior to the caudal alae. The width of the head across the appendages is 0.04 mm. The buccal capsule is 0.032 mm. long and the anterior muscular and posterior glandular portions of oesophagus measure about 0.38 and 1.6 mm., respectively. The nerve ring lies at about 0.26 to 0.3 mm. from the anterior end,

The hind end of the worm is provided with large alae and there is a set of large pedunculated papillae comprising two pairs of precloacal, a double papilla immediately behind the cloaca, and two pairs of postcloacal papillae. The spicules are very unequal, the long one measuring 2.9 to 3.05 mm. and the short one 0.95 to 1 mm. long. The long and the short spicules have a thickness of 0.006 to 0.008 mm. and 0.012 to 0.0125 mm. respectively. The long spicule tapers before ending in a fine point while the short and thick spicule is truncated at the posterior end. The cloaca is situated about 0.11 mm. from the tail end.

Females measure 17 to 25 mm. long and have a breadth of 0.175 to 0.2 mm. at about the middle. The head end measures about 0.045 mm. across the appendages. The buccal capsule is 0.035 to 0.045 mm. long and the anterior muscular and the posterior glandular portions of oesophagus measure 0.41 to 0.45 mm. and 1.74 to 1.775 mm. long, respectively. The nerve ring lies 0.31 to 0.35 mm. from the anterior end. The vulva is rather inconspicuous and is situated at about 3.8 to 4.9 mm. from the anterior end, or, at 1.375 to 2.8 mm. posterior to the termination of oesophagus. The tail tapers obliquely behind the anus to end in a rounded tip and measures 0.12 to 0.13 mm. long. The eggs which are thick at the sides and thin at the extremities contain a fully formed larva and measure 0.048 to 0.05 mm. long and 0.028 to 0.03 mm. broad.

Host : The Indian Red-wattled Lapwing, *Lobivanellus indicus indicus.*

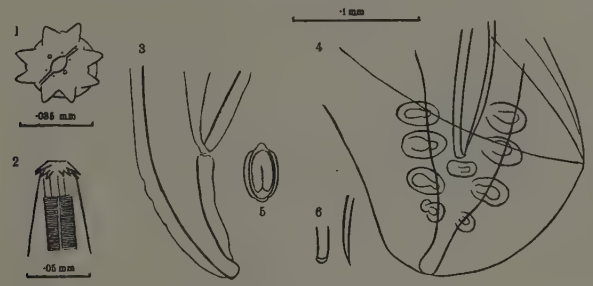
Location : Gizzard.

Locality : Sheikhupura-Punjab.

Relationships

Gil'Bert (1930) created the genus *Stellocaronema* for the species, *Stellocaronema skrjabini*, which he described from the material collected from *Hydrochelidon nigra* and *Vanellus vanellus* during the 22nd and 30th Helminthological Expeditions to the Western territory of U.S.S.R. *S. buckleyi* possesses all the essential characters of the genus such as the four pairs of tooth-like cuticular appendages at the head end and the vulva being situated at about the end of the first third of the body. It differs from the only other species of the genus, *Stellocaronema skrjabini*, in the length of the spicules and the number and arrangement of caudal papillae. The spicules in *S. skrjabini* are reported by Gil'Bert to measure 2.012 to 2.18 and 0.55 to 0.67 mm. long respectively while in *S. buckleyi* they measure

2.9 to 3.05 mm. and 0.95 to 1 mm. long. The lengths of the spicules were found to be fairly constant in the ten male specimens in which they were measured. Gil'Bert (1930) described three preanal and two postanal pairs of caudal papillae in *S. skrjabini*. In *S. buckleyi* there are two pairs of preanal, two pairs of postanal and a double papilla just posterior to the anus.



Stellocaronema buckleyi, n.sp.

Fig. 1.—End face view of head. Fig. 2.—Anterior end. Fig. 3.—Tailend of female. Fig. 4.—Caudal extremity of male. Fig. 5.—Egg. Fig. 6.—Posterior ends of spicules.

SPIRURIDAE Oerley, 1885
Habronematinae Chitwood and Wehr, 1932
Hadjelia truncata (Creplin, 1825)

This species is known from a large number of birds and has been recorded by Singh (1948) from the Indian Roller (*Coracias benghalensis*) from Hyderabad-Deccan, India. Chabaud and Campana (1950) have redescribed the cephalic extremity of the parasite and suggest that *Hadjelia inermis* is a synonym of *H. truncata*. Singh (1948) has recorded *H. inermis* from the White-breasted Kingfisher (*Halcyon smyrnensis*) from Hyderabad Deccan. The description of *H. inermis* as given by Singh (1948) suggests that he had been dealing with immature material. He describes the eggs of *H. truncata* as oval, thick shelled and embryonated, measuring $52\mu \times 34\mu$ while in his *H. inermis* he found the eggs to be unsegmented and apparently unfertilized, measuring $18\mu \times 13\mu$. Again, Singh (1948) does not mention the lengths of the spicules in his *H. inermis* and merely states that the left spicule is six times longer than the right. This ratio is the same as in *H. truncata*.

The specimen in the author's collection was recovered from the proventriculus of an Indian Magpie (*Pica Pica*) which died in the Zoological Gardens, Lahore. *Pica Pica* has not previously been recorded as a host for this species. The measurements of the male specimen are as follows :

Body length 7 mm.; body width just before the caudal alae 0.165 mm.; width at the head end 0.026 mm.; length of buccal capsule 0.05 mm.; nerve ring at a distance of 0.19 mm. from the anterior end ; the anterior muscular and posterior glandular portions of the oesophagus are 0.33 mm. and 2.1 mm. long, respectively and the spicules are 0.3 mm. and 1.45 mm. long, respectively.

Host : Indian Magpie, *Pica Pica*.

Location : Proventriculus.

Locality : Zoological Gardens, Lahore-Punjab.

ACUARIIDAE Seurat, 1913

Acuariinae Railliet, Henry and Sissof, 1912

Dispharynx spiralis (Molin, 1858)

The parasite is known from all over the world and has been recorded from a large number of birds. Singh (1948) recorded the species from jungle crow (*Corvus macrorhynchus*) from Hyderabad-Deccan, India. Baylis (1939) described the parasite in detail from a fowl from Ceylon, and from a Bronze-winged Jacana (*Metopidius indicus*) from the Zoological Gardens, Calcutta. The parasite has been mentioned by Edminster (1947) as the most significant of the helminth parasites of the Ruffed Grouse. The species is recorded here from a collection from a Magpie (*Pica Pica*) which died in the Zoological Gardens, Lahore. The magpie has not previously been recorded as a host for this species. The measurements of a male and a female specimen are as follows:

Male : Body length 3.6 mm.; maximum body width 0.19 mm.; pharynx 0.115 mm. long ; nerve ring 0.21 mm. from anterior extremity ; oesophagus 1.4 mm. long ; distance of posterior edges of cordons from the anterior end 0.29 mm.

Female : Body length 5.5 mm.; maximum body width 0.3mm.; nerve ring 0.38 mm. from the anterior end ; pharynx 0.12 mm. long ; anterior muscular and posterior glandular portions of oesophagus 0.625 and 1.5 mm. long, respectively ; distance of

posterior edges of cordons from anterior end 0.5 mm.; length of tail 0.15 mm.

Host : Indian Magpie, *Pica Pica.*

Location : Gizzard.

Locality : Zoological Gardens, Lahore-Punjab.

Acuaria anthuris (Rud : 1819) Railliet, Henry and Sissof, 1912

The species has been recorded from a large number of birds in Europe and Asia and it has been described in detail by Maplestone (1931). In India, the species has been recorded by Maplestone (1931) under the name *Acuaria scutata* from the Red-billed Chough (*Pyrrhocorax pyrrhocorax*) and the Indian Tree Pie (*Dendrocitta rufa*). Baylis and Daubney (1922) and Singh (1948) recorded the species from the Red-billed Blue Magpie (*Urocissa melanocephala occipitalis*), and the House Crow (*Corvus splendens*) and the Jungle Crow (*Corvus macrorhynchus*), respectively. The species is recorded here from a Rosy Pastor shot at Sheikhupura. The worms, females only, were found with their head buried in the musculature of the gizzard. The Rosy Pastor is recorded as a new host for the species. The body measurements of the specimens fall within the range of those of Maplestone (1931).

Host : The Rosy Pastor or Rose coloured Starling, *Pastor roseus.*

Location : Gizzard.

Locality : Sheikhupura-Punjab.

Acuaria brevispicula Maplestone, 1932

Maplestone, 1932 described this species from a single male specimen from a Magpie Robin (*Copsychus saularis*) from the Zoological Gardens, Calcutta. The author obtained a collection of this species, comprising twelve males and seven females, from an Indian Mynah (*Acridotheres ginginianus*) shot at Sheikhupura. A detailed description of the species is being presented because of certain discrepancies in Maplestone's description apparently due to the inadequacy of the material at his disposal.

The worms have triangular lips and the cordons end just anterior to the termination of oesophagus in the male and a short distance anterior to the vulva in the female.

Male : measures 4.5 to 5.6 mm. long and has a maximum thickness of 0.13 to 0.18 mm. The pharynx measures 0.125 to 0.16 mm. long and the anterior muscular and posterior glandular portions of the oesophagus measure 0.26 to 0.38 mm. and 0.95 to 1.1 mm long, respectively. The nerve ring lies at a distance of about 0.225 mm. from the anterior extremity. The body tapers behind the cloaca which lies at a distance of 0.17 to 0.19 mm. from the tail end. The caudal alae are composed of peripheral thick and median thin portions. There are twelve pairs of caudal papillae comprising four preanal and eight postanal pairs. The papillae, except the last postanal pair, have long and thin peduncles but small pulps. The spicules which are more or less equal and similar, measure 0.1 to 0.12 mm. long.

Females : measure 13.5 to 14.5 mm. long and have a maximum thickness of 0.35 to 0.4 mm. The pharynx is 0.2 to 0.25 mm. long and the anterior muscular and posterior glandular portions of the oesophagus measure 0.72 to 0.78 and 2.2 to 2.5 mm. long, respectively. The vulva is situated slightly behind the middle of the body at a distance of about 3.9 mm. behind the termination of oesophagus or 7.5 to 7.8 mm. from the anterior end. The eggs are thick shelled and measure 0.039 to 0.04 × 0.023 to 0.025 mm.

The determination of the exact number of papillae in the male could be done only by a searching examination and in some specimens the first and the fourth postanal pair were particularly difficult to make out. In one of the specimens two pairs of minute papillae instead of one were observed at the tip of the tail.

The configuration of the spicules was observed to be an interesting feature in the species. The spicules which are more or less equal and similar give in most specimens an erroneous impression of their being unequal and dissimilar, (Figs. 7–12), on account of differences in their location inside the body and their state of protrusion outside the cloaca in different specimens. Since the spicules are very difficult to roll, the possibility of determining their shape and size from a small amount of material is rather limited. *Acuaria kungi* described by Singh (1948) from two males and one female specimens agrees in description with that of *A. brevispicula* as described above, the shapes of the spicules in *A. kungi* falling within the range of those drawn in Figs. 7–12. *A. kungi* (Singh, 1948) is apparently identical with *A. brevispicula.*

Host : Indian Mynah (*Acridotheres ginginianus*).

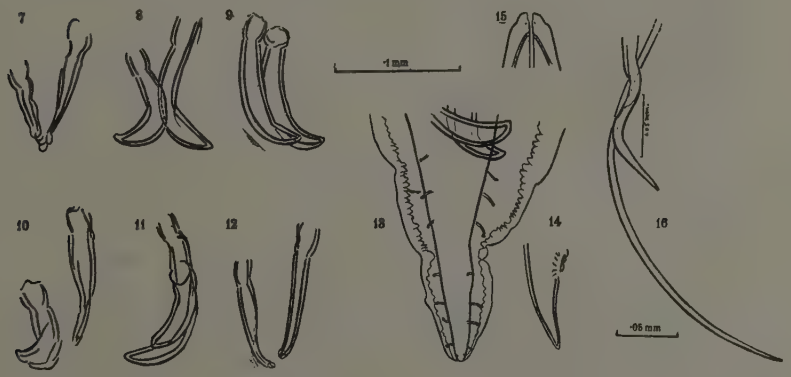
Location : Under the horny layer of gizzard.

Locality : Sheikhupura, Punjab-Pakistan.

FILARIIDAE Claus, 1885
Diplotriaeninae Skrjabin, 1916
Diplotriaena nochti Hoeppli and Hsu, 1929

The species was recovered from two specimens of the Rosy Pastor (*Pastor roseus*) and the Indian Mynah (*Acridotheres ginginianus*) shot at Sheikhupura. The specimens measured as follows :

Male : Body length 46 to 52 mm.; tridents 0.135 to 0.16 mm. long ; anterior muscular and posterior glandular portions of oesophagus, respectively, 0.29 to 0.38 and 6.0 to 7.2 mm. long ; nerve ring at a distance of 0.2 to 0.24 mm. from the anterior end and the two spicules 2.325 to 3.0 and 0.59 to 0.65 mm. long, respectively.



Acuaria brevispicula
Figs. 7–12.—Spicules in different forms of male. Fig. 14.—Tail end of female. Fig. 13.—Caudal end Fig. 15.—Anterior end.

Diplotriaena nochti
Fig. 16.—Spicules.

Female : Body length 140 to 150 mm.; tridents 0.15 to 0.17 mm. long ; the anterior muscular and posterior glandular portions of oesophagus 0.35 to 0.42 and 8.5 to 9.2 mm. long, respectively ; the vulva at a distance of 0.58 to 0.64 mm. from the anterior end and the eggs about 0.045 × 0.03 mm.

The species was originally described by Hoeppli and Hsu (1929) from Crested Mynah (*Acridotheres crystallus*) and was later recorded by Sandground (1933) from *Aethiopsar crystatellus* from Indo-China. Though the species has not been recorded in Indo-Pakistan, yet

the descriptions of the other *Diplotriaena* species recorded from the subcontinent suggest quite plainly that *D. nochti* has been confused, in India, with other species. Canavan (1931) recorded *Diplotriaena tricuspis* from the Indian Mynah (*Acridotheres ginginianus*) and Columbian Jay (*Cyanocorax affinis*) from the Philadelphian Zoological Gardens. He has fortunately, stated the measurements of the specimens from the two hosts separately and while the measurements from specimens from Columbian Jay conform to the description of *D. tricuspis*, the measurements of specimens from the Indian Mynah are undoubtedly related to *D. nochti*. *D. tricuspis* was recorded again, by Mazhar (1933) from Aligarh District, United Provinces, India. The description of *D. tricuspis* as recorded by Mazhar fully corresponds with that of *D. nochti* Heoppli and Hsu, 1929. It is interesting that Mazhar did notice the discrepancy in his identification when he pointed out that, "as it is apparent that the measurements of *D. tricuspis* as given by Boulenger (1928) and Li (1933) for the species are comparatively very small". Since both the above authors have not mentioned *D. nochti* as a synonym of *D. tricuspis*, it is apparent that they had overlooked the description of *D. nochti*.

Karve (1934) described a new species, *Diplotriaena acridotherei*, from *Acridotheres tristis* from Central Provinces, India. The description of this species corresponds in all essential details with that of *D. nochti* and as Karve (1934) does not provide a differential diagnosis of this species, his reasons for calling it a new species are unknown. Karve (1934), at the same time, described another new species, *Diplotriaena nagpurensis* from a single male specimen from the same host. The description of this species, again, agrees with that of *D. nochti* except that the caudal papillae could not be found in this specimen. The presence or absence of caudal papillae has not been given much importance in this genus; Boulenger (1928) states that they are notoriously difficult to discern, and their number as described by different authors for the same species sometimes differs in a striking manner. Thus, Schmerling (1925) failed to find papillae in his *D. artemisiana* while Boulenger (1928) recorded three or possibly four pairs of preanal and at least one pair of postanal papillae. Sandground (1933) in his account of *D. artemisiana* and *D. nochti* stated that "on first examination the caudal extremity appeared to have no papillae but prolonged study revealed quite definitely a series of minute and elusive structures". In the earlier accounts of *D. tricuspis* (Fedschenko, 1874) male caudal papillae were said to be absent. According to Sandground (1933), the number and arrangement of papillae on the tail of the male is difficult to

use for purposes of taxonomy. In addition, Karve (1934) mentioned the presence of a black pigment at the caudal extremity in *D. nagpurensis* and it is possible that this pigment obscured the papillae in the specimen. Sandground (1933) has also mentioned the adhering of foreign matter to the tail of the specimens, which made discerment of papillae difficult. Since *D. nagpurensis* agrees in all other details with *D. nochti*, the two species are considered here to be identical.

The genus *Diplotriaena* is at present in much need of study. About fifty species have been described and many investigators have commented that the genus needs a thorough revision. Many authors have described species without attempting even a partial differential diagnosis and sufficient information on the variability of the characters in the genus is lacking. Seibert (1944), commenting on the reliability of the characters in the genus states that the least variable factors appear to be the lengths of the spicules and the tridents. The position of the tridents is, however, expected to vary greatly in relation to the anterior end because of the motility of these organs. In the light of the known factors, it is, in many cases, extremely difficult to judge the identity of certain species. For instance, it is not quite clear as to how *Diplotriaena conceptionis*, described by Caballero (1948) from *Dives dives*, differs from *D. artemisiana* Schmerling (1925).

Summary

1. A new species, *Stellocaronema buckleyi*, is described from the Indian Red-wattled Lapwing (*Lobivanellus indicus indicus*) from Sheikhupura district of the Punjab province of Pakistan.

2. The following species are recorded from new hosts and for the first time from Pakistan : *Hadjelia truncata* and *Dispharynx spiralis* from the Indian Magpie (*Pica pica*) ; *Acuaria anthuris* from the Rosy Pastor (*Pastor roseus*) ; *Acuaria brevispicula* and *Diplotriaena nochti* from the Indian Mynah (*Acridotheres ginginianus*).

3. It is contended that *Diplotriaena tricuspis* of Canavan (1931) and Mazhar (1933) are identical with *Diplotriaena nochti*, and that *Diplotriaena acridotheres* (Karve, 1934) and *Diplotriaena nagpurensis* (Karve, 1934) should also be treated as synonyms of *D. nochti*.

4. *Acuaria brevispicula* Maplestone, 1932 has been redescribed and certain discrepancies in Maplestone's description, due to insufficient material, have been made good. It is contended that *A. kungi* Singh, 1948 should fall as a synonym to *A. brevispicula*.

References

Baylis, H. A., 1939.—"The Fauna of British India, including Ceylon and Burma." Nematoda. Vol. 2. London.

Baylis, H. A. and Daubney, R., 1922.—"Report on the parasitic nematodes in the collection of the Zoological Survey of India." *Mem. Indian Mus.*, **7,** 263–374. (W.L. 13401)

Boulenger, C. L., 1928.—"Report on a collection of parasitic nematodes, mainly from Egypt. Part V. Filarioidea." *Parasitology*, **20,** 32–55. (W.L. 16035)

Caballero, Y. C., 1948.—"Etudes helminthologiques sur la vallée du Rio Papaloapan (Mexique). II. Quelques filaires de bactraciens et d'oiseaux." *Ann. Parasit. hum. Comp.*, **23,** 323–333. (W.L. 899a)

Chabaud, G. Alain and Campana, Y., 1950.—"Note sur le genre *Hadjelia* Seurat 1916 (Nematodes-Spiruridae)." *Ann. Parasit. hum. Comp.*, **25,** 435–440. (W.L. 899a)

Edminster, F. C., 1947.—The ruffed grouse. Its life story, ecology and management. Macmillan Co. XXXVI. pp. 385.

Gil'Bert, L. I., 1930.—"Zur Fauna der Vogelnematoden des Westgebiets USSR." (Russian text; German summary). *Nauch. Izv. Smolensk.* V., 6, 91–112. (W.L. 14945b)

Hoeppli, R. and Hsu, H. F., 1929.—"Helminthologische Beiträge aus Fukien und Chekiang. Part II. Parasitische Nematoden aus Vogeln und einem Tummler." *Arch. Schiffs-u. Tropenhyg.* **33,** 24–34. (W.L. 1804)

Karve, J. N., 1934.—"Two new species of the genus Diplotriaena (Nematoda) parasitic in the common Indian myna (*Acridotheres tristis tristis*). *J. Univ. Bombay.*, **2,** 75–81. (W.L. 11594c)

Li, H., 1933.—"Report on a collection of parasitic nematodes, mainly from North China. Part I. Filarioidea. *Parasitology.*, **25,** 192–223. (W.L. 16035)

Maplestone, P. A., 1931.—"Parasitic nematodes obtained from animals dying in the Calcutta Zoological Gardens." *Rec. Indian Mus.*, **33,** 71–171. (W.L. 17746)

———— 1932.—"Parasitic nematodes obtained from animals dying in the Calcutta Zoological Gardens." *ibidem*, **34,** 229–261. (W.L. 17746)

Mazhar, A. K., 1933.—"On some parasitic nematodes of Aligarh district." *Z. parasitenk.* **6,** 269–272. (W.L. 23536b)

Sandground, J. H., 1933.—"Report on the nematode parasites collected by the Kelley-Roosevelt Expeditions to Indo-China with descriptions of several new species. Part I. Parasites of birds." *Z. Parasitenk.*, **5,** 543–564. (W.L. 23536a)

Schmerling, A. A., 1925.—"Zur Kenntnis der Vogelfilarien (*Squamofilaria* n.g. und *Diplotriaena artemisiana* n.sp.) von *Coracias garrula.* L." *Zbl. Bakt. Abt.* 11., **63,** 267–271 (W.L. 23684).

Seibert, H. C., 1944.—"Notes on the genus *Diplotriaena* with the description of a new species." *Trans. Amer. micr. Soc.*, **53,** 244–253. (W.L. 21400)

Singh, S. N., 1948.—"Studies on the helminth parasites of birds in Hyderabad State. Nematoda II." *J. Helminth.*, **22,** 199–218. (W.L. 11224c)

Journal of Helminthology, Vol. XXX, *Nos.* 2/3, 1956, *pp.* 113–118.

A New Echinostome Trematode, *Pameileenia gambiensis* gen. et sp. nov. from the Intestine of a Colubrid Snake in West Africa

By C. A. Wright and S. R. Smithers

About thirty echinostome flukes were found in the intestine of a snake, *Grayia tholloni* Mocquard, killed at a washing place in the Kotu stream about ten miles west of Bathurst, Gambia in March 1955.

The flukes were concentrated in a portion of the intestine about 2 in. long, slightly behind the middle of its length. The intestinal wall was strongly inflamed and thickened in this region, the thickening being evident on the outer surface of the gut even before it was opened. The worms were very firmly attached to the gut wall and attempts to remove them by force merely resulted in mechanical damage. The piece of intestine concerned was cut out and placed in Ringer's solution in a refrigerator. After about two hours the flukes released their hold and were easily removed. They were fixed in formal-acetic, a few under light cover-slip pressure, and permanent preparations were stained in aceto-carmine.

The body of the parasite is elongate, five or six times as long as broad, dorso-ventrally flattened and widest just behind the testes, roughly at the junction of the third and last quarters of the body length. The length varies between 5 and 6 mm. in mature, unpressed specimens and the maximum width between 0.8 and 1.0 mm.

The anterior end is developed into a typical echinostome head-collar which bears twenty-seven spines of approximately uniform size, 0.10–0.11 mm. in length. They are arranged dorsally in a

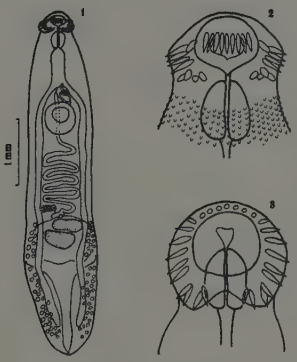
single, uninterrupted row while ventrally there is, on either side of the pharynx, a group of four corner spines. Anteriorly the cuticle is covered with close-set scales in a quincuncial arrangement. The anterior limit of these scales is on a level with the middle of the pharynx and they extend backwards in dense formation to the region of the ventral sucker. The area immediately surrounding the genital pore appears to be devoid of scales. From the level of the ventral sucker backwards they thin out considerably, are very sparse at the level of the ovary and practically absent, even on the dorsal surface, behind the testes. The ventral sucker is large and powerful, about 0.5 mm. in diameter, and is situated at approximately the junction of the first and second quarters of the body length.

The oral sucker is approximately half the size of the ventral and is subterminal in position. In unpressed specimens its dimensions vary between 0.24×0.24 mm.—0.27×0.26 mm. There is a short prepharynx and the pharynx is large and barrel-shaped or slightly pyriform, measuring about 0.20×0.23 mm. The oesophagus, which is wide, runs back and divides just in front of the ventral sucker. The two simple intestinal caeca extend back almost to the posterior end of the body.

The excretory vesicle is Y-shaped, the division of the arms occurring just behind the testes. The anterior extent of the arms has not been seen but they reach at least as far forward as the ventral sucker. The excretory pore is terminal.

The testes are large and smooth in outline, roughly ovoid, with their long axes in the transverse plane. They lie slightly obliquely one behind the other in the posterior part of the middle third of the body length. The vasa efferentia unite a short distance in front of the anterior testis. The vas deferens runs forward slightly to the right of the mid-line then crosses over toward the left side dorsal to the ventral sucker. The cirrus sac lies just in front of the ventral sucker, overlapping it slightly, either in the mid line or slightly to one side of it. The sac contains the vesicula seminalis, a somewhat flask-shaped organ, the proximal part of which is large and thin walled while the distal part is narrower and tubular with a thicker wall. The cirrus is unarmed. The genital pore lies in the mid-line ventral to the bifurcation of the intestinal caeca. The ovary is much smaller than the testes and is also ovoid and transversely elongate. It lies just in front of the testes on the right side. The oviduct

leaves the ovary on its anterior side and runs back to Mehlis' gland which lies between the ovary and anterior testis. Ventral to Mehlis' gland lies the vitelline reservoir into which run the two transverse vitelline ducts. The vitelline glands extend from almost the posterior region of the body to the level of the anterior testis and the ducts run slightly forward and towards the reservoir from the anterior limit of the follicles. Opening from the side of Mehlis' gland nearest to the mid-line is the uterus, the proximal part of which contains spermatozoa and acts as a receptaculum seminis uterinum. The uterus



Pameileenia gambiensis gen. et sp.nov.

Fig. 1.—Ventral view of whole worm. Fig. 2.—Ventral view of head.

Fig. 3.—*En face* view of head crown.

runs forward, coiled between the intestinal caeca. Just before it reaches the level of the ventral sucker it ceases to coil and runs straight forward in the mid-line to the genital pore. The eggs are large, 0.095–0.11 mm. × 0.45–0.55 mm., thin shelled and apparently without an operculum. Those in the more distal part of the uterus are embryonated and contain a patch of dark pigment granules similar to a miracidial eyespot. It is not known whether under normal conditions the eggs are fully embryonated and ready to hatch on leaving the host or not. The extreme distal part of the uterus is without eggs except in some of the pressed specimens, in

one of which there is an egg containing an advanced embryo actually lodged in the genital pore. It is possible that the prefixation treatment of cooling in Ringer's solution brought about the discharge of any mature eggs present in the uteri of the worms. In the specimen mentioned above the advanced but not fully mature egg seen in the genital pore may have been forced forward from lower down the uterus by coverslip pressure.

Note on the host

Mr. J. C. Battersby to whom we are indebted for the identification of the host tells us that *Grayia tholloni* has hitherto been recorded in the literature only from the Anglo-Egyptian Sudan southwards to Katanga and thence westwards to the French Congo. There is a specimen from Northern Nigeria in the collection of the British Museum. Dr. H. W. Parker has supplied the following information : "The distributional gap between the present record from the Gambia and the Anglo-Egyptian Sudan is partly bridged by the specimen from Northern Nigeria and it is therefore probable that the species has a completely circum—Rain Forest distribution. Its distribution relative to the Rain Forest form *Grayia caesar* seems to be paralleled by that of *G. smythia* relative to *G. ornata*, the latter being also a forest species".

Grayia tholloni is a water snake and feeds mainly on fish.

Discussion

This fluke is of interest because it is the first definite record of an echinostome from a snake. Odhner (1902) described *Cotylotretus rugosus* from a Brazilian snake *Coluber pullatus* (*Spilotes pullatus*). Mendheim (1943) does not include this species in his work on the Echinostomatidae, nor is it mentioned by Dietz (1910), but anatomically there seem to be every reason to include it in this family, despite the lack of spines on the head collar.

The new fluke falls readily into the sub-family Echinostomatinae in which the cirrus pouch is restricted to the region in front of the ventral sucker and the collar spines are uninterrupted dorsally. It is distinguished from the other genera in this sub-family by the extent of the vitelline glands and by the embryonated eggs. One other Echinostome genus is described as having embryonated eggs,

Pelmatostomum Dietz, 1910. The new form can easily be distinguished from this by the absence of a dorsal cleft in the head collar and the limited posterior extent of the cirrus pouch.

The marked inflammation of the intestinal wall of the snake suggests the possibility that this host is perhaps not the normal one. *Grayia tholloni* is a fish-eating water snake and it is conceivable that this specimen had eaten a fish infected with metacercariae of a fluke normally parasitizing a fish-eating bird. It is significant that all other twenty-seven spined Echinostomes are parasites of fish-eating birds or mammals. Since a drop in temperature is probably one of the principal factors initiating embryonation of echinostome eggs when they are passed from their warm-blooded hosts, development in a cold-blooded reptile might account for the embryonation of the eggs in this form.

Pameileenia gen. nov.

Echinostomatinae of medium size with a head collar bearing an uninterrupted single row of spines of which the corner spines are in a group of four on either side. The male genital system comprises two testes and a cirrus pouch with a flask-shaped vesicula seminalis and an unarmed cirrus confined in front of the ventral sucker. The female system consists of an ovary, Mehlis' gland and receptaculum seminis uterinus; the vitelline glands extend from almost the posterior end of the body to the level of the anterior testis. The eggs in the uterus appear to lack an operculum and are embryonated.

Pameileenia gambiensis n.sp.

With the characters of the genus and twenty-seven collar spines. The type material has been deposited in the British Museum (Natural History), B.M. coll. nos. 1955.10.10.1–8.

Summary

A new echinostome fluke *Pameileenia gambiensis* gen. et sp. nov. is described from a water snake *Grayia tholloni* from Gambia. A note on the distribution of the host is included since this is the first record of it from the Senegambia region.

ACKNOWLEDGMENTS

The authors are indebted to Dr. H. W. Parker and Mr. J. C. Battersby for information about the host and to Mr. S. Prudhoe for advice and criticisms on the helminthological aspects of this work.

REFERENCES

DIETZ, E., 1910.—"Die Echinostomiden der Vögel." *Zool. Jber.*, Suppl. 12, 256–512. (W.L. 23835.)

MENDHEIM, H., 1943.—"Beiträge zur Systematik und Biologie der Familie Echinostomatidae." *Arch. Naturgesch.*, Leipzig (N.F.), **12,** 175–302. (W.L. 1782.)

ODHNER, T., 1902.—"Trematoden aus Reptilien." *Öfvers. Vetensk Akad. Förh., Stockh.*, 1902, No. 1. Stockholm, 19–46. (W.L. 15615.)

REES, GWENDOLEN, 1939.—"Studies on the germ-cell cycle of the digenetic trematode *Parorchis acanthus* Nicoll. Part I. *Parasitology*, **31,** 417–433. (W.L. 16035.)

———1940.—Studies on the germ-cell cycle of the digenetic trematode *Parorchis acanthus* Nicoll. Part II. *ibid.*, **32,** 372–391.

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A Technique for the Detection of *Polystoma integerrimum* in the Common Frog *(Rana temporaria)*

By J. B. Jennings, B.Sc., Ph.D.
Department of Zoology, The University of Leeds.

An infection of the frog bladder by the Monogenetic Trematode *Polystoma integerrimum* can be detected by testing the urine for the presence of the pigment haematin. This substance is an end product of the digestion of blood, which the parasite sucks from the capillaries of the bladder wall, and it is voided from the flatworm's gut into the bladder to pass eventually to the exterior with the urine (Jennings—unpublished work).

An alkaline solution of luminol (3-amino pthallic cyclic hydrazide hydrochloride) and hydrogen peroxide exhibits an intense blue luminescence in the presence of haematin (Proescher & Moody, 1938) and this reaction, which is very sensitive, can be used to detect the pigment in the urine of infected frogs.

The urine is collected in a $2\frac{1}{2}$ in. by $\frac{1}{4}$ in. tube by gently squeezing the frog. If there is no yield or it is inadequate, a further collection can be made one to two hours later. The tube containing the urine is inserted into the top of a light-proof box and viewed through a hole in the side as a small amount of the luminol-peroxide reagent (an 0·1% solution of luminol in 5% aqueous sodium carbonate, plus one-fifth its volume of 3% hydrogen peroxide) is added. If haematin is present an intense blue luminescence develops which is easily distinguished from the faint luminescence normally exhibited by the reagent.

Faecal contamination of the urine must be avoided since the peroxidases of the faecal protozoa react with luminol-peroxide to give a false positive reaction. The chances of contamination can be reduced by depriving the frogs of food during the course of the examination.

Polystoma does not give out a continuous discharge of haematin so that the urine of an infected frog may well give a negative reaction. It has been found, however, that this difficulty can be overcome by testing the urine at twenty-four hour intervals over a period of five days—when at least one positive reaction will be obtained if the parasite is present. Of 219 frogs tested in this way 172 gave five consecutive negative urine tests and proved to be uninfected on subsequent post mortem examination. The remaining forty-seven frogs gave at least one positive urine reaction and examination showed that forty-four of these were infected with *Polystoma*. The other three frogs were uninfected and their positive tests were probably due to faecal contamination.

An attempt to simplify the procedure by isolating frogs in individual jars and testing the liquid in these every twenty-four hours was unsuccessful. Faecal contamination always occurred, although starved frogs were used, and false positive reactions were obtained even after boiling the liquid to eliminate protozoan peroxidases. These false positives were due possibly to bile pigments in the faeces since bile reacts strongly with luminol-peroxide and the reaction is not destroyed by boiling.

Other Trematodes (*Gorgodera* and *Gorgoderina*) may occur in the frog bladder but they do not produce haematin during digestion. Thus the occurrence of haematin in the urine is conclusive proof of the presence of *Polystoma* in the bladder and hence infected frogs can be detected and isolated for the provision and maintenance of a stock of this parasite in the laboratory.

I am grateful to Professor E. A. Spaul, Dr. T. Kerr, and Dr. R. W. Owen for their advice and help during the development of this technique.

Reference

Proescher, F. and Moody, A. M., 1938.—"The detection of blood by chemiluminescence." *J. Lab. clin. Med.*, **24**, 1183. (W.L. 11285)

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* The Use of Di-*n*-Butyl Tin Dilaurate for Treatment of Chickens Experimentally Infected with *Davainea proglottina*

By A. H. Abdou, B.V.Sc., Ph.D.

From the Parasitology Department,
London School of Hygiene and Tropical Medicine

Up to the present time no taeniacidal agent has been established on the basis of adequate evidence as satisfactory for the removal of tapeworms, especially *Davainea proglottina*, from poultry. Tapeworms are generally regarded as responsible for considerable damage to the poultry industry. The principal remedies that have been recommended are areca nut, turpentine, oil of chenopodium, extract of male fern, Kamala, pomegranate root bark, arecolin hydrobromide and thymol.

Guberlet (1916) recommended the lye-in-grain treatment, a mixture of grains and lye, for the removal of tapeworms from poultry which showed symptoms of this infection. Later Wickware (1921), Riley and James (1922) stated that the lye treatment is not of value for tapeworms. Hall and Foster (1918) using oil of turpentine (2 cc.) and areca nut (1 grain) obtained no satisfactory results in expelling tapeworms from the intestine of fowls, but some of these parasites were removed after giving a dose of 0.2 ml. of oil of chenopodium (0.4 ml. per kilo. body weight). They remarked that none of the substances tested hitherto for tapeworms in poultry have proved very satisfactory. Wickware (1921) tested pomegranate root bark (dosage 1.5–6.0 grains) but in all cases tapeworms were still present in moderate to large numbers. He also attempted to expel tapeworms parasitizing fowl, with male fern extracts (2 drams and 9 days later 1–1.5 drams), thymol (1–6 grains) and areca nut (30–60 grains) but none of these agents proved effective. Later Hall and Shillinger (1926) using male fern, reported better results, but their attempts to remove the tapeworms with arecoline hydrobromide (1/32–1/4 grain) were unsuccessful; Kamala (1 gram) was found to be a suitable drug for removing the tapeworms.

Using tetrachlorethylene (dose 2.0 cc.) Schlingman (1926) removed one tapeworm from a fowl but admitted that he did not have sufficient data to confirm this result.

Patterson (1929) recommended 30–60 minims of oil of turpentine with castor oil as an efficient taeniacide for poultry.

* Part of a Thesis approved by the University of London for the award of the Ph.D. degree.

Bisset (1926) treated a considerable number of emaciated fowls, heavily infected with *Davainea proglottina* by introducing a teaspoonful of turpentine into the crop, followed by a purge. The parasites were expelled from the intestine in large numbers and the expulsion resulted in a marked improvement in the condition of these birds.

Bayon (1930) recommended thymol in one or two grains doses at five day intervals until the segments were no longer passed in the faeces. Later (1933) he stated that equal parts of oil of turpentine and olive oil (2.0 cc. for an average size adult bird) administered at weekly intervals gave satisfactory results.

Stafseth (1953) using "Iodine vermicide" on *Raillietina cesticillus* and *Hymenolepis carioca* was able to remove only the strobilae while the scolices remained unaffected; a second or third dosing was often necessary to kill young stages which may have been located deep in mucosa, between the villi.

Levine (1938) in his studies on the control of the poultry cestode *D. proglottina* in the United States tried treating infected chickens with Kamala (1.0 gm.), areca nut and Kamala (2.0 grms. and 0.5 gm.), oil of turpentine (5.0 cc.), carbon tetrachloride (2.0 cc.), tetrachlorethylene (2.0 cc.), arecoline hydrobromide (0.25 grain), thymol (2.0 gms.), oil of chenopodium (0.2 cc.), rotenone (1.2 gm.), pelletierine tannate (0.8 gm.), hexylresorcinol (0.4 gm.), santonin (1.0 gm.), betanaphthol (1.0 gm.), "iodine vermicide" (30 cc.), gen-sal worm powder (2–3 gms.), thymol-nicotine tablets, Kamala nicotine tablets and nota caps (1.45 gm.) but in no instance was the treatment successful. (The weight of birds used ranged from 2.75 to 3.25 pounds). Ground areca nut was the only drug in the whole series to remove any scolices. This drug when tried on two infected birds removed 14% of the scolices present in the first bird and 0.7% of those present in the second.

Acharya (1939) claimed to have obtained excellent results in removing both nematodes and cestodes from poultry using 10% solution of colloidal iodine in water, the solution being injected straight into the gizzard (1 oz. of the solution for birds weighing 4 lb.). Worms expelled were *Ascaridia lineata*, *Raillietina tetragona* and *Cotugnia digonopora*. Guthrie and Harwood (1948) stated that phenyl mercuric phthalate is to some extent effective against *Davainea proglottina*; it removed 49.8% of the tapeworms present in twenty chickens.

Generally, however, although experiments have been carried out with a large number of drugs for one reason or another, none of these compounds has been acceptable for use in the field.

During the last few years different tin compounds have been reported as being effective in removing tapeworms from poultry. The use of tin compounds is briefly reviewed as follows :

Tin is probably one of the oldest metals used in medicine ; as early as 1589 Paracelsus remarked upon the efficacy of tin compounds as anthelmintics. However, it was not until 1899 that their use as taenicidal agents was confirmed by Dommes. Lepinay (1933) reported that when pure oxide of tin was administered to dogs large numbers of tapeworms including the heads were removed. The same treatment was applied to a human being and resulted in the expulsion of *Taenia saginata*. Recently Guthrie and Harwood (1941) and Guthrie, Powick and Blandell (1944) reported that tin compounds have anthelmintic value, especially when mixed with a little synthetic pelletierine hydrochloride ; tin oleate, stannous oxalate and stannous tartrate removed *R. cesticillus*, *R. tetragona* and *H. carioca* ; and Kerr (1951) stated that a tetravalent tin compound possesses anthelmintic properties against the poultry cestode *R. cesticillus*. The work of both Kerr and Guthrie and his co-workers thus shows that consideration should be given to the use of tin compounds. Accordingly, preliminary work was carried out by me with di-*n*-butyl tin dilaurate to test its efficacy as a taeniacidal agent against *D. proglottina*. This compound is an oily liquid, and was used as supplied by the Tin Research Institute. For the purposes of simplicity the name Butynorate for this compound will be used throughout this work.

Material and Methods

The procedure followed involved the controlled infection of chickens by the administration of a known number of fully matured cysticercoids obtained from experimentally infected slugs of different species. Each bird received about 500 cysticercoids administered orally. This method produced reasonably constant infection in the birds.

All birds used in this experiment (cockerels) were bought at eight weeks old from a well-established hatchery in St. Albans, Hertfordshire, where the birds were reared hygienically and were never allowed free range. The birds were kept by me in individual cages and checked for natural infection with *Davainea proglottina* by examination of the droppings for the presence of any gravid segments. This was done twice weekly for six weeks. At no time were any segments discovered and it was felt quite certain that, before the experiment, these birds were free from this infection. The birds were brown Leghorn crossed with light Sussex and

all came from one and the same hatch. They were weighed at the beginning of and at intervals during this experiment. The birds were fed on a well balanced diet (growers pellets) containing barley mash, fish meal, maize, wheat and minerals.

After being infected, each bird was confined to a separate compartment of a laying battery. Each of these compartments had a slotted floor through which the droppings passed into a container kept wet with water. The 24-hour droppings of each birds were daily examined for the presence of the gravid segments of *Davainea proglottina*.

On the 11th day seven of these birds passed proglottides, on the 12th day proglottides were recovered from another six birds and the remaining bird passed segments on the 13th day.

Toxicity test

In order to determine the maximum dose of Butynorate which could be tolerated by chickens, four birds of about three to four months old were first used.

Two birds were each given 0.5 ml. of the compound per kilo. body weight. The drug was administered in hard gelatin capsules. Each capsule was pushed well down into the oesophagus to make quite certain that the bird swallowed it.

Bird No. 1 weighed 1,050 grams and received 1.0 ml. of Butynorate. Bird No. 2 weighed 1,445 grams and received 1.5 ml. of Butynorate. The other two birds (Nos. 3 and 4) were each given 1.0 ml. of the drug per kilo. body weight. Each of these birds weighed 2,005 grams.

Each bird was then put in a separate cage for observation, and given the normal diet. No marked decrease in appetite was noticed in any of the birds and the only noticeable departure from normal was that the droppings were liquified for the next few days, after which the faeces regained their normal condition.

In the next experiment four other birds were used. Their exact age was not definitely known but all were about one year old.

	Dose Kgm. Wt.
Bird No. 5 weighed 3,600 grams and received 5.4 ml. of Butynorate	1.5 ml.
Bird No. 6 weighed 3,410 grams and received 4 ml. of Butynorate	1 ml.
Bird No. 7 weighed 3,010 grams and received 3 ml. of Butynorate	0.9 ml.
Bird No. 8 weighed 2,670 grams and received 2 ml. of Butynorate	0.7 ml.

All birds were subjected to the same conditions and each was kept in a separate cage. Again, the only result noticed was that watery droppings were passed for the 48 hours after medication, but the bird which had received 5.4 mls. appeared off colour for a week, and died later. All the other birds appeared normal and their appetites did not seem to be reduced.

Result : Although these experiments were carried out on eight birds only, the results indicate that medication of chickens with Butynorate is relatively non toxic. The maximum tolerated dose is approximately 1.0 ml. per kilo. body weight.

Medication of chickens as a single dose by capsule

The droppings of the birds selected for the first experiment were examined for proglottides. Before treatment was commenced all the birds were passing gravid segments.

After 24 hours fasting the drug contained in a hard gelatin capsule was administered to each bird. The bird was held tightly with its mouth widely open, then the capsule containing the measured amount of the drug was put at the back of the bird's mouth and gently pushed down its oesophagus. The capsule could be felt through the oesophagus on its way to the crop. It was found best to give the drug in millilitres per kilogram body weight. This avoided the necessity of testing groups of birds of the same weight. The balancing of the groups to weight would have required a large number of infected birds. The weight of birds used ranged from 2.24 to 2.87 kilograms. Care was taken to ensure that the birds did not eject the capsules. Two hours after treatment the regular food was placed before the birds.

The birds were killed 14 days after medication, a period of time which permitted development of additional proglottides from any scolices which might remain, thus facilitating the detection of the worm at post-mortem. Each bird was weighed on the day of treatment and on the 14th day before destruction for examination.

Experiment I

Four birds were used in this experiment. Only two of them were given the drug, a dose of 1.0 ml. per kilo. body weight. The other two acted as controls (See Table I). They were subjected to the same conditions as the treated birds except that the gelatin capsules administered were empty. Two weeks after treatment each bird was necropsied and the intestinal tract carefully examined for any remaining *Davainea proglottina*.

Result : No *Davainea proglottina* or their gravid segments were detected in the two treated birds, while a very large number (300–400) of these tapeworms was recovered from the duodenum of the birds acting as controls. Having found that the drug had a definite effect as a taeniacidal agent against *Davainea proglottina*, it was decided to ascertain the minimum effective dose.

TABLE I

No. of bird	Dose per kilo body wt.	Weight of bird (gm.)	Dose given	Weight after 14 days (gm.)	Result
180	1.0 ml.	2690	2.7 ml.	2740	No *D. proglottina* found
181	1.0 ml.	2450	2.5 ml.	2515	No *D. proglottina* found
34	Control	2360	0	2425	Many adult *D. proglottina* found in duodenum and gravid segments in rest of gut
35	Control	2495	0	2585	

EXPERIMENT II

In this experiment thirteen experimentally infected birds were used. The dose varied (see Table II) from 1.0 ml. to 0.06 ml. per kilo. body weight. Ten birds were treated, the other three, (group 6, untreated), acting as controls. Ten of these birds were killed exactly 14 days after treatment and the gut of each bird was examined for the presence of *Davainea proglottina*. The remaining three birds (Nos. 190 and 200, group 4 and 5 respectively and No. 193, group 6) were treated again as will be mentioned later.

Result : No evidence was found to indicate the presence of *D. proglottina* in the gut of the birds which received 1.0 ml. or 0.5 ml. of Butynorate per kilogram body weight. But the worm was present in both birds of group 3 and in one bird in each group 4 and 5, (Nos. 194 and 201 respectively). The worms found in the control group 6 (300–400), were far more numerous than those in groups 3, 4 and 5, (100–200). It was, therefore, decided not to kill the remaining two birds in groups 4 and 5 but to treat them again using a larger quantity of the drug.

Table II

Group	No. of bird	Dose per kilo body wt.	Weight of bird (gm.)	Dose given (ml.)	Weight after 14 days (gm.)	Result
1	192	1 ml.	2240	2.25	2350	No *D. proglottina* or gravid segments found
	197		2430	2.45	2515	
2	196	0.5 ml.	2800	1.35	2870	No *D. proglottina* or gravid segments found
	195		2420	1.25	2450	
3	184	0.25 ml.	2270	0.6	2350	Both *D. proglottina* and gravid segments recovered
	191		2750	0.7	2835	
4	194	0.12 ml.	2700	0.3	2650	Both *D. prgglottina* and gravid segments recovered
	190		2520	0.3	2590	Not killed
5	201	0.06 ml.	2670	0.15	2763	Both *D. proglottina* and gravid segments recovered
	200		2575	0.15	2655	Not killed
6	185	Control	2260	0	2350	Large numbers of adult *D. proglottina* found in duodenum and gravid segments in intestine
	189	Control	2590	0	2665	
	193	Control	2365	0	2420	Not killed

Experiment III

Bird No. 190 received 2 ml. Butynorate in gelatin capsules.
Bird No. 200 received 2 ml. Butynorate in gelatin capsules.
Bird No. 193 received empty capsules and acted as control.

Result: All three birds were killed 14 days later and while no *Davainea proglottina* was present in the gut of birds Nos. 190 and 200, the duodenum of bird No. 193 (control) contained large numbers of this worm (about 300).

Conclusion

The results of these experiments using di-*n*-butyl tin dilaurate with chickens experimentally infected with *Davainea proglottina*, indicate that this compound possesses anthelmintic value and that, in view of the results obtained, investigations on similar lines but on a larger scale are desirable.

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REFERENCES

ACHARYA, S. K., 1939.—"Experimental treatment of helminth parasitic infection of poultry with colloidal iodine or iodine vermicide." *Indian Vet. J.* **16,** 83–93. (W.L. 9959.)

BAYON, H. P., 1930.—"Common and preventable parasitic ailments of the domestic fowl. A contribution to the study of the hygiene of poultry." *Vet. Rec.*, **10,** 1129–1142. (W.L. 22523.)

———1944.—"Worms and how to treat them." *Poult. Fmg.*, Lond, 110 (2858). (W.L. 16410c.)

GUBERLET, J. E., 1916.—"Studies on the transmission and prevention of cestode infection in chickens." *J. Amer. vet. med. Ass.*, **49,** 218–237. (W.L. 11022.)

GUTHRIE, J. E., and HARWOOD, P. D., 1941.—"Use of tin preparations for the treatment of chickens experimentally infected with tapeworms." *Amer. J. vet. Res.*, **2,** 108–116. (W.L. 628a.)

——— 1944.—"Limited tests of mixtures of tin oleate with ammonium compounds for the removal of experimental tapeworm infections of chickens." *Proc. helm. Soc. Wash.*, **11,** 45–48. (W.L. 16747a.)

———1948.—"Phenyl mercuric compounds for the removal of tapeworms from poultry." *J. Parasit.*, **34** (6, Sec. 2), Suppl., 15. (W.L. 11428.)

GUTHRIE, J. E., POWICK, W. C. & BANDEL, D., 1941.—"Critical tests with tetra-alkyl tin compounds for the removal of *Raillietina cesticillus* from experimentally infected chickens." *N. Amer. Vet.*, **22,** 22–24. (W.L. 15224.)

HALL, M. C. and FOSTER, W. D., 1918.—"Efficacy of some anthelmintics." *J. agric. Res.*, **12,** 397–447. (W.L. 10966.)

HALL, M. C. and SHILLINGER, J. E., 1926.—"Kamala, a satisfactory anthelmintic for tapeworms in poultry." *N. Amer. Vet.*, **7,** 51–58. (W.L. 15224.)

KERR, K. B., 1948.—"Hexachlorophene as an agent for the removal of *Raillietina cesticillus*." *Poult. Sci.*, **27,** 781–788. (W.L. 16421.)

LEVINE, P. P., 1938.—"Studies on the control of the poultry cestode *Davainea proglottina* (Dav.)." *Cornell Vet.*, **28,** 220–227. (W.L. 6888.)

PATTERSON, F. D., 1929.—"Common intestinal parasites of poultry." *Vet. Med.*, **24,** 21–23. (W.L. 22520.)

RILEY, W. A. & JAMES, L. G., 1922.—"Life history and methods of control of the chicken nematode (*Heterakis papillosa* Bloch)." *Rep. Minn. agric. Exp. Sta.* 30th (1921–22), 70–71. (W.L. 18375a.)

SCHLINGMAN, A. S., 1926.—"Miscellaneous tests of tetrachlorethylene, a new anthelmintic." *J. Amer. vet. med. Ass.*, **68,** 741–754. (W.L. 11022.)

WICKWARE, A. B., 1921.—"Studies on the use of anthelmintics in fowls." ibid., 59, 731–740.

Journal of Helminthology, Vol. XXX, *Nos.* 2/3, 1956, *pp.* 129–142.

Studies on the Serological Response in Sheep to Naturally Acquired Gastro-intestinal Nematodes

I. Preparation of Antigens and Evaluation of Serological Techniques

By E. J. L. Soulsby, M.A. (Cantab.), Ph.D., M.R.C.V.S., D.V.S.M.
Department of Animal Pathology, University of Cambridge

Immunological reactions to helminth infestation have been studied extensively in recent years and in some instances have become valuable aids in the diagnosis of helminth disease. In many experimental infestations it has been possible to correlate an increased antibody content of the blood with an increased resistance to infestation. With respect to sheep nematodes Stewart (1950a,b,c,d,) has shown with *Haemonchus contortus* and *Trichostrongylus spp.* that a complement-fixing-antibody response occurs following infestation and that this is stimulated following the administration of infective larvae *per os*. Stewart demonstrated a general correlation between the fall of egg counts and the rise in antibody titre in experimentally infected animals and in field studies the percentage of sheep maintaining or increasing antibody titre was inversely proportional to the percentage maintaining or increasing egg count.

Sera from immune animals have been shown to cause precipitates at the physiological orifices of larvae when they are placed in homologous immune sera. This phenomenon has been demonstrated with the larvae of sheep trichostrongyles (Hawkins & Cole, 1945), *Nippostrongylus muris* (Sarles & Taliaferro, 1936; Sarles, 1938), *Trichinella spiralis* (Oliver-Gonzalez, 1940, 1941; Roth, 1945; Mauss, 1940), Hookworms (Otto, 1940) and *Ascaris lumbricoides* (Oliver-Gonzalez, 1943). Antibodies causing these oral, anal and excretory pore precipitates are considered by Oliver-Gonzalez (1946a) to be "functional" in type, that is, antibodies which contribute materially to the phenomenon of resistance, and that their production is stimulated by the metabolic products present in the secretions and excretions of the larvae.

It is reasonable to assume that where circulating antibody can be correlated with increased resistance to infestation then a proportion of that antibody is of the "functional" type. Other "non-functional" antibodies occur and have been demonstrated by Oliver-Gonzalez (*loc. cit.*) in experimental *Ascaris* infestation. These play no obvious part in resistance to infestation. It is very probable that similar antibodies are elaborated in other nematode infestations.

It would appear desirable, therefore, when determining the immune status of a parasitized animal by serological techniques to utilise tests which will demonstrate "functional" antibody and to use antigens which have stimulated the production of this functional antibody, i.e. "functional" antigens. Of the tests available for the estimation of antibody the quantitative precipitin analytical method of Heidelberger and Kendall (1935) is probably the most reliable and strictly quantitative. Owing to the involved technique of this test and to the relatively low levels of serum antibody in helminth infestation it is not applicable to routine testing of serum samples. The complement fixation test is used frequently, but suffers from several disadvantages, one of which is the occurrence of non-specific reactions with various heterologous antigenic substances (Mackie and Finkelstein, 1928). The generally adopted procedure of the use of complete or 100 per cent haemolysis as an end point does not afford a sensistive index of activity since the relationship of lysis of cells to units of complement is not linear (Wadsworth, Maltaner and Maltaner, 1931, 1938). Furthermore, complement fixing antibodies in sheep naturally infested with nematodes are seen to be comparatively low (Steward, *vide supra*) and periods may occur when the test is negative.

In an attempt to demonstrate circulating antibody in high titre and eliminate periods of negative serological reaction the passive haemagglutination test described by Boyden (1951a) was investigated and was found to be satisfactory as a means of demonstrating antibodies to helminth infestation.

In the preparation of antigens in this work an attempt was made to obtain "functional" antigen and to preserve as far as possible the natural character of the antigens obtained from living worms. In contrast to the methods of other workers (Stewart, *vide supra*; Dunn, 1954) who used heat in the preparation of antigen, in this investigation antigens were prepared at low temperatures. The use

of low temperatures was prompted by the work of Oliver-Gonzalez (1943) who demonstrated that differences in the antigenic structure of the tissues of *A. lumbricoides* could be detected when these were obtained by low temperature techniques.

Part I. Material and Methods

Antigens. These were prepared from living worms which were obtained from freshly killed sheep or cattle. The contents of the abomasum and small intestine were washed through a copper sieve and when sufficiently clean the contents were placed in petri dishes and the worms were collected manually and placed in the following groups :—

1. Abomasal parasites (H.C.), (containing *H. contortus*, *Ostertagia* and *Trichostrongylus spp.*).

2. Intestinal Nematodes (I.N.), (containing *Trichostrongylus spp.*, *Cooperia curticei*, *Chabertia ovina*).

3. *Cooperia oncophora* (C.O.B.).

4. *Bunostomum spp.* (B.P.I.), (containing *B. phlebotomum* and *B. trigonocephalum*).

These were dried lightly with filter paper and placed in solid CO_2 at –79°C. as soon as possible after collection. When sufficient material had been collected the weight of the frozen worms was determined by differential weighing and the material was then subjected to alternate thawing and freezing by allowing the material to thaw at 4°C. for several hours and then remain frozen for twenty hours. This was repeated five times with the object of allowing the ice particles forming inside the cells of the parasite to disrupt the cells and release the protein constituents. After the final freezing the material was ground from the frozen state to a fine emulsion in a sterile mortar, sterile saline being added to the emulsion in the proportion of one gramme of original frozen worm material to 4 mls. of saline. The emulsion was allowed to stand at 4°C. for two hours, after which it was centrifuged for thirty minutes at 6,000 r.p.m. The particulate free, opalescent supernatant was removed and stored in 5 ml. aliquots in solid CO_2.

An extract of whole female *A. lumbricoides* was prepared in the same manner.

Preparation of Antisera

Antisera against the intestinal nematodes (I.N.) *Cooperia oncophora* (C.O.B.) and *A. lumbricoides* extracts were prepared in rabbits by intravenous injection on five occasions at weekly intervals. Serum was obtained from blood taken by intracardiac puncture, the serum being stored at –40°C. Serum from sheep naturally infected with nematodes was separate from blood obtained by vena puncture of the jugular at weekly intervals and blood for serum from worm free lambs was obtained by intracardiac puncture of lambs born by Caeserian section and before they had suckled their mothers.

The Complement Fixation Test

In the examination of the relationships of the artificially produced immune sera the complete haemolysis end point technique was employed using 2.5 minimal haemolytic units of complement per tube. The volume of each reagent was 0.5 ml., the final volume of reagents being 2.0 ml. Sheep erythrocytes used in the test were collected and preserved in modified Alsever's Solution (Bukantz, Rein and Kent, 1946). All sera were inactivated at 56°C. for thirty minutes, and antibody titres were determined by serial dilution of the sera in saline starting at 1 in 5.

Antigens were examined for anticomplementary action and, with the exception of the H.C. antigen, were weakly anticomplementary in the undiluted state. Dilution to 1 in 2 eliminated this but to be within safe limits a dilution of 1 in 4 in saline was adopted. With the H.C. extract there was a weak anticomplementary action at a dilution of 1 in 8 ; this antigen was used in a dilution of 1 in 32.

Relationships between Rabbit Antisera

At the outset of this investigation it was intended to examine the relationships between antisera against sheep helminths and *A. lumbricoides*, since some cross reaction between these had been noted by Stewart (1950a). If this proved to be of a high order then it was hoped that *A. lumbricoides* could be used as a ready and plentiful supply of non-specific antigen. In addition to this, it was desirable to know which species antigen, amongst the sheep nematodes, gave the best reactions with homologous and related heterologous antiserum. These relationships are presented in Table I. Rabbit No. 1 died during the course of immunization which was repeated in rabbit No. 2.

TABLE I

Relationships between sera from rabbits immunised with sheep nematodes and the homologous and heterologous antigens.

	Rabbit No. 1 Immunised with Antigen I.N.						Rabbit No. 2 Immunised with Antigen I.N.							Rabbit No. 3 Immunised with Antigen C.O.B.							Rabbit No. 4 Immunised with *Ascaris* Antigen					
Serum dilutions	1/5	1/10	1/20	1/40	1/80	1/160	1/5	1/10	1/20	1/40	1/80	1/160	1/320	1/5	1/10	1/20	1/40	1/80	1/160	1/320	1/5	1/10	1/20	1/40	1/80	1/160
Antigen																										
B.P.I.	***	***	**	–	–	–	***	***	***	**	–	–	–	***	***	***	***	–	–	–	***	***	***	–	–	–
I.N.	***	***	***	**	–	–	***	***	***	***	**	–	–	Not tested.							Not tested.					
C.O.B.	***	***	***	***	**	–	***	***	***	***	***	***	–	***	***	***	***	***	**	–	***	***	***	**	–	–
Ascaris	**	*	–	–	–	–	**	*	–	–	–	–	–	***	***	**	–	–	–	–	***	***	***	**	–	–

*** Complete inhibition of haemolysis.
** Slight haemolysis (*c.* 10%–20%).
* Marked haemolysis (*c.* 60%).
– Complete haemolysis.

The serum of a non-immunised buck rabbit was found to have a surprisingly high titre, as is shown in Table II.

TABLE II

Antibody content of serum of non-immunised rabbit

Antigen	Serum Dilution						
	1/5	1/10	1/20	1/40	1/80	1/160	1/320
C.O.B.	***	***	**	–	–	–	–
B.P.I.	***	*	–	–	–	–	–
I.N.	***	**	*	–	–	–	–

(Degrees of haemolysis are indicated in Table I.)

The faeces of the rabbit referred to in Table II contained 200 strongyle eggs per gramme and it is assumed that this animal demonstrated antibody to an infestation by *Trichostrongylus retortaeformis* since this parasite was found to predominate in the laboratory stock. The examination of further sera from stock rabbits showed that antibodies were present in all the sera examined but only in the low titre of 1 in 5.

It is seen from these results that, with the exception of *Ascaris*, a cross reaction existed between the antigens from different groups of parasites and that a relatively close relationship existed between them. The use of *Ascaris* as a non-specific antigen in detecting antibodies to intestinal nematodes of sheep was precluded since titres obtained with this antigen and heterologous immune sera were low. It would appear that the *Ascaris* extract is deficient in antigens common to the *Trichostrongylidae* of sheep yet possessing a similar basic antigenic pattern to the sheep parasites. In these rabbit immunisations it is seen that *Cooperia oncophora* proved most efficient, the *Bunostomum* antigen being poorest. Nevertheless it was an efficient antigen. Dunn (1954) has demonstrated that *B. phlebotomum* antigen is satisfactory and easily obtainable: these results indicate the *Cooperia* antigen is even more efficient but owing to its smaller size it may not be so readily obtainable as *B. phlebotomum.*

The abomasal parasite (H.C.) antigen was available later. It was used in a dilution of 1 in 32 in comparison with the *Cooperia* antigen in normal dilution, the results being shown in Table III.

TABLE III

Comparison of the Abomasal Parasites and *Cooperia* Antigen
Serum of Sheep 85 Dilution

	1/5	1/10	1/20	1/40	1/80
C.O.B. Antigen	***	***	**	–	–
H.C. Antigen	***	*	–	–	–

It is seen from Table III that in a dilution of 1 in 32 an antigen containing adult *H. contortus* is active with the serum of a naturally infested sheep.

Since proof had been obtained that the antigens prepared were active, that they gave substantial reactions with homologous and heterologous immune sera and since large quantities of antigen were required for future tests the separate antigens were pooled. This pooled antigen, in a dilution of 1 in 32, was tested by the complement fixation technique against sera taken from sheep naturally infested with nematodes, the results being presented in Table IV.

TABLE IV

Sheep Sera and date collected

	G32 15.1.53	G32 22.1.53	G32 29.1.53	G32 5.2.53	G32 26.2.53	B850 1.10.53	B850 8.10.53
Dilution at which positive	1/10	1/20	1/5	1/10	1/5	1/40	1/10

Table IV indicates that the pooled antigen proved satisfactory in detecting antibodies in naturally infested sheep.

Part II

The Passive Haemagglutination Test (*Boyden*, 1951)

The materials and methods employed followed the description given by Boyden with the following exception.

Serum Diluents. In Boyden's original work a 1 in 250 dilution of rabbit serum was employed for washing tanned cells treated with antigen and a 1 in 100 dilution for diluting test sera. He indicated that with certain antigens the protective action of normal rabbit serum on erythrocytes was not necessary. In this investigation, however, autoagglutination of treated cells occurred where buffered saline alone was used ; some protection against autoagglutination of treated erythrocytes was therefore required. Sera from stock rabbits was used in preliminary tests but agglutination of the treated cells occurred. Since it has been shown in Part I that normal stock rabbits contain antibodies against helminth antigens it was concluded that antibodies in the diluent sera were reacting specifically with the antigen coated red cells and causing them to haemagglutinate. It was found, however, that preserved guinea-pig serum obtained from Messrs. Burroughs Wellcome had no such action and served as a satisfactory diluent for test sera in a dilution of 1 in 100 in saline (pH 7.2) and as a washing agent after antigen treatment in a dilution of 1 in 250. At these complement dilutions slight haemolysis occurred in the lowest dilutions of strong antisera but was not observed during treatment of red cells.

Tannic Acid. A 1 in 40,000 dilution of "Reagent" Grade Tannic Acid in buffered saline (pH 7.2) was found to be most satisfactory. Fresh solutions were made up daily for use in the tests.

Antigen. The pooled antigen referred to in Part I was diluted to 1 in 40 in saline buffered at pH 6.4.

The dilutions of test sera in 0.5 ml. quantities were made in one millilitre concavities on perspex W.H.O. influenza agglutination plates, adequate controls being set up with each set of tests. Two drops from a Pasteur pipette (approx. 0.1 ml.) of the treated erythrocytes were placed in each serum dilution and mixed by means of a glass rod. The racks were left at room temperature for two hours when the resulting degree of haemagglutination was recorded.

It was found to be essential that the various stages of treatment were carried out accurately and as a continuous process, so that all batches of sensitised cells produced had undergone identical periods

of incubation and treatment. Failure to do this resulted in a variation of the final titre with individual sera.

Results of the Test. The effect of absorption of sera with packed erythrocytes was investigated to ascertain if normal blood iso-agglutinin antibodies elevated the titre of the sera. The results are presented in Table V.

TABLE V

Titres with absorbed and non-absorbed sera
Pooled Nematode Antigen

Sera		Serum Dilutions								
		1/5	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
Serum A	α*	****	***	**	*	–	–	–	–	–
	β*	****	***	*	–	–	–	–	–	–
Serum B†	α	****	****	****	****	****	***	***	***	**
	β	****	***	***	***	***	***	**	–	–
Serum Sheep A411	α	****	***	*	–	–	–	–	–	–
	β	****	*	–	–	–	–	–	–	–
Serum Sheep A81	α	****	***	***	***	***	***	**	–	–
	β	****	***	***	***	**	–	–	–	–
Serum Worm Free Lamb	α	*	–	–	–	–	–	–	–	–
	β	–	–	–	–	–	–	–	–	–

α* Unabsorbed Sera. β* Sera absorbed with an equal volume of packed red cells for 10 minutes.

† Serum B. Serum from rabbit immunised with *A. lumbricoides.*

It will be observed that the absorption of sera with red cells reduces the titre of the haemagglutination and indicates that isoagglutinins against the stroma of the red cells can cause unwanted haemagglutination and must be removed before the specific test can be carried out.

The marked reaction obtained with the anti-*Ascaris* serum was unexpected and will be commented on later. The serum from a worm-free lamb was negative after absorption.

Tests were set up to see if these absorbed antisera had any effect on untreated cells. All the results were negative.

The test was then employed as a routine method for the examination of sera from sheep for antibodies against gastro-intestinal helminths. In all tests serum from a worm-free lamb was employed as a negative control and on all occasions it failed to show any reactions. One serum sample showed a positive titre of 1 in 5120 and this was used as a known positive, the titre remaining high throughout the period of use.

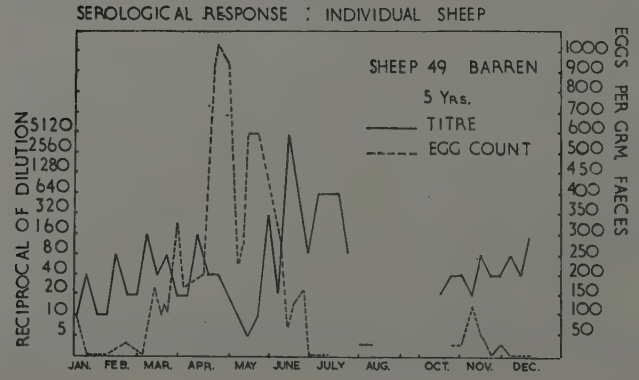
Examples of the serological titres in some sheep over a period of months are given in Graphs I to III. In certain sheep a comparison was made between the titres of the complement fixation test and the passive haemagglutination technique. These are shown in Graph IV.

Discussion

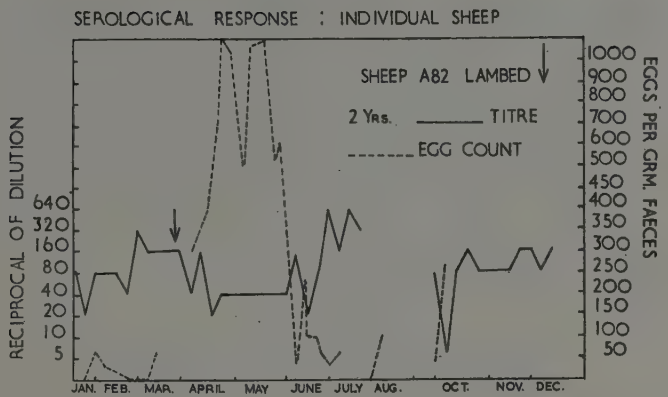
For the fulfilment of the passive haemagglutination technique it appears necessary to use protein antigen for the treatment of tanned cells (Boyden, 1951 ; Grabar, Boyden, Taquet and Borduas, 1952). It has been shown in Part I that there is a satisfactory cross reaction between the protein antigens from sheep nematodes so that the use of any of these antigens would have been satisfactory. This type of antigen has been shown to be of use in complement fixation tests on sheep which indicates that protein antigens are satisfactory in demonstrating anti-sheep nematode antibodies. It is believed that the antigens prepared in this investigation are most likely to contain the antigens which, in the host, stimulate "functional" antibodies.

Graphs I to III give examples of the serological response in three sheep naturally infested with gastro-intestinal nematodes over a period of a year. In these sheep there is the general tendency for the antibody titre to rise after the egg counts have fallen, thus being in accord with the work of Stewart (*vide supra*). All the sheep showed the "Spring rise" phenomenon and it is noticeable that the

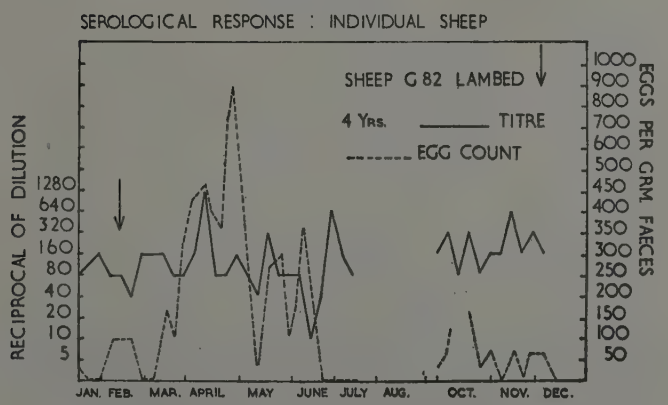
GRAPH I



GRAPH II



GRAPH III



termination of the "Spring rise" is accompanied by a marked rise in antibody titre thus suggesting that immunological reactions play some part in the "Spring rise". It is of further interest that there is a general rise of antibody previous to the "Spring rise" which would suggest an increased stimulation of immune responses by an increased acquisition of larvae or activity of larvae already acquired.

Graph IV gives a comparison between titres obtained by the passive haemagglutination and the complement fixation techniques and shows that not only are the titres higher with the former test but also that there is no constant relationship between the two titres. Borduas and Grabar (1953) have demonstrated a similar relationship between precipitin titres and passive haemagglutination titres with anti-protein antibodies and have suggested that the latter test may detect incomplete or "univalent" antibody. However, Coombs and Fiset (1954) have shown that the Boyden technique does not detect incomplete antibody. As yet no proof exists as to the existence of incomplete antibodies in helminth infestations but further study of this aspect may lead to more fundamental knowledge regarding immunity to helminth infestations.

GRAPH IV

COMPARISON BETWEEN TITRES OF COMPLEMENT FIXATION AND PASSIVE HAEMAGGLUTINATION TESTS.

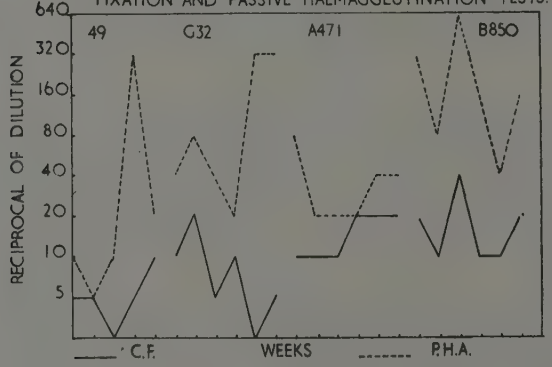


Table V indicates that in the passive haemagglutination technique the absorption of isoagglutinins from test sera is essential in order to preclude false reactions. The marked reaction of the rabbit anti-*Ascaris* serum is interesting. Absorption of this serum, though greatly reducing the titre, still left a substantial agglutination reaction. This may be due to natural incompatibility of rabbit sera with sheep cells but which is usually removed by absorption. The production in the rabbit of antibodies which are closely allied to and react with blood group substances of sheep red cells may be another explanation. The production in the rabbit of such antibodies following infection or injection with *A. lumbricoides* has been demonstrated by Oliver-Gonzalez (1946b).

SUMMARY

Antigens were prepared by low temperature methods to preserve "functional" antigen. These antigens showed marked cross reactivity and were satisfactory in complement fixation tests.

The passive haemagglutination test using "functional" antigen gave titres which were higher than those obtained with the complement fixation test. High antibody titres were observed in sheep naturally infested with nematodes and a relationship between antibody titre and egg count was demonstrated.

ACKNOWLEDGMENT

I wish to thank Dr. H. D. Crofton, Bristol University, for the data relating to the weekly egg counts.

REFERENCES

BORDUAS, A. G. and GRABAR, P., 1953.—"L'hémagglutination passive dans la recherche des anticorps antiprotéiques." *Ann. Inst. Pasteur.*, **84,** 903. (W.L. 857).

BOYDEN, S. V., 1951a.—"The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera." *J. Exp. Med.*, **93,** 107. (W.L. 11189).

BOYDEN, S. V., 1951b.—"Bacterial haemagglutination and allied subjects." Ph.D. Thesis. Cambridge.

BUKANTZ, S. C., REIN, C. R. and KENT, J. F., 1946—"Studies in Complement Fixation. II. Preservation of sheep's blood in citrate dextrose mixtures (modified Alsevers solution) for use in the complement fixation reaction." *J. Lab. Clin. Med.*, **31,** 394. (W.L. 11285).

COOMBS, R. R. A. and FISET, M. L., 1954.—"Detection of complete and incomplete antibodies to egg albumen by means of a sheep red cell-egg albumen antigen unit." *Brit. J. exp. Path.*, **35,** 472. (W.L. 3566).

DUNN, A. M., 1954.—"The preparation of helminth antigen from fresh *Bunostomum trigonocephalum*." *J. Helminth.*, **28,** 9. (W.L. 11224C).

GRABAR, P., BOYDEN, S. V., TAQUET, A. and BORDUAS, A. G., 1952.—"Mise en évidence de deux anticorps différents dans les sérums tuberculeux par hémagglutination passive." *C. R. Acad. Sci.*, **234**, 899. (W.L. 6628).

HAWKINS, P. A. and COLE, C. L., 1945.—"Studies on sheep parasites. V. Immunity to gastro-intestinal nematodes." *J. Parasit.*, **31,** 113. (W.L. 11428).

HEIDELBERGER, M. and KENDALL, F. E., 1935.—"A quantitative theory of the precipitin reaction. III. The reaction between crystalline egg albumen and its homologous antibody." *J. exp. Med.*, **62,** 697. (W.L. 11189).

MACKIE, T. J., and FINKELSTEIN, M. H., 1928.—"A study of non-specific complement fixation with particular reference to the interaction of normal serum and certain non-antigenic substances." *J. Hyg., Camb.*, **28,** 172. (W.L. 11233).

MAUSS, E. A., 1940.—"The *in vitro* effect of immune serum upon *Trichinella spiralis* larvae." *Amer. J. Hyg.*, **32,** D, 80. (W.L. 600a)

OLIVER-GONZALEZ, J., 1940.—"*In vitro* action of immune serum on larvae and adults of *Trichinella spiralis*." *J. infect. Dis.*, **67,** 292. (W.L. 11250)

———— 1941.—"The dual antibody basis of acquired immunity in Trichinosis." *ibid.*, **69,** 254.

———— 1943.—"Antigenic analysis of the isolated tissue and body fluids of the roundworm *Ascaris lumbricoides var. suum*." *ibid.*, **72,** 202.

———— 1946.—"Functional antigens in helminths." *ibid.*, **78,** 232.

———— 1946.—"Immunological relationships among polysaccharides from various infectious organisms." *ibid.*, **79,** 221.

OTTO, G. F., 1940.—"A serum antibody in dog actively immunized against the hookworm, *A. caninum*." *Amer. J. Hyg.*, **31,** D, 23. (W.L. 600a)

ROTH, H., 1945.—"Serodiagnosis of Trichinosis by microscopically testing with living Trichinae larvae." *Nature, Lond.*, **155,** 785. (W.L. 14900)

SARLES, M. P., 1938.—"*In vitro* action of immune rat serum on the nematode *Nippostrongylus muris*." *J. infect. Dis.*, **62,** 337. (W.L. 11250)

SARLES, M. P., and TALLIAFERRO, W. H., 1936.—"The local points of defense and the passive transfer of acquired immunity to *Nippostrongylus muris*." *ibid.*, **59,** 207.

STEWART, D. F., 1950a.—"Studies on resistance of sheep to infestation with *Haemonchus contortus* and *Trichostrongylus spp.* and on the immunological reactions of sheep exposed to infestation. I. The preparation of antigens for the complement fixation test and the reactivity of the biochemical fractions of *H. contortus*." *Australian J. Agric. Res.*, **1,** 285. (W.L. 2244a)

———— 1950b.—"II. The antibody response to infestation with *H. contortus*." *ibid.*, **1,** 301.

———— 1950c.—"III. The antibody response to infestation with *Trichostrongylus spp.*" *ibid.*, **1,** 413.

———— 1950d.—"IV. The antibody response to natural infestation and the 'self cure' phenomenon." *ibid.*, **1,** 427.

WADSWORTH, A., MALTANER, E. and MALTANER, F., 1931.—"The quantitative determination of the fixation of complement by immune serum and antigen." *J. Immunol.*, **21,** 313. (W.L. 11238)

———— 1938.—"Quantitative studies of the reaction of complement fixation with tuberculous immune serume and antigen." *ibid.*, **35,** 93.

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Damage to Henbane by Stem Eelworm *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936

By E. B. Brown
National Agricultural Advisory Service, Cambridge

During November 1953 some specimens of henbane plants, *Hyoscyamus niger*, were received from Long Melford, Suffolk. On examination these plants were found to be infested with *Ditylenchus dipsaci*. According to the farmer, henbane is a difficult crop to grow on the farm and it is possible that *D. dipsaci* may have been the cause of previous trouble, although another pest, the henbane flea beetle, *Psylliodes hyoscyami* L., is also prevalent in the area. Henbane is a biennial and is cultivated for medicinal use. Three acres on this field were ploughed up in 1953 because of failure due to eelworm damage.

Infested plants were stunted and the leaf bases swollen (Plate I). Many plants were rotting just above soil level and the majority of the outer leaves were dying off. The symptoms were very similar to the typical "tulip root" condition of severely-attacked oats.

Various crops were sown in pots filled with sterilized soil to which infested henbane material had been added, to determine the race of *D. dipsaci* attacking the henbane. Onions, field beans and S147 Oats developed symptoms but broad red clover remained free from attack. The oat race, which is a common and widespread pest, was therefore shown to be the cause of the damage. Henbane seedlings grown in infested soil quickly developed characteristic swelling of the hypocotyl. This field grew oats in the previous year and henbane in 1948 but no damage to either crop was noticed by the farmer.

A small seed sample left over after drilling was obtained for examination and twenty grammes of seed yielded four eelworms. As the seed was only slightly infested it is probable that the infestation originated mainly from the soil.

Henbane has not previously been recorded as a host of *D. dipsaci*.

I wish to thank Mr. W. E. Dant for the photograph.

Left : Henbane attacked by *D. dipsaci*. Right : Healthy plant.

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A Seed-Borne Attack of Chrysanthemum Eelworm *(Aphelenchoides ritzema-bosi)* on the Annual Aster *(Callistephus chinensis)*

By E. B. Brown
National Agricultural Advisory Service, Cambridge

In early May 1954 some annual aster plants (*Callistephus chinensis*) being grown in sterilized compost in seed boxes were received from a nursery at Mildenhall, Suffolk. Many of these plants were severely stunted and malformed, and some were completely dead. At first sight it looked as if the centres of these plants had been eaten out, but subsequent examination revealed a heavy infestation of chrysanthemum eelworm (*Aphelenchoides ritzema-bosi*), living ectoparasitically among the young leaves. Bunches of nematodes were easily visible in the leaf axils under the low power of a binocular microscope, or even under a good hand lens.

The leaves in the centres of these plants were much stunted and swollen. In many leaves the lamina was completely absent and in others it was reduced, the leaf becoming long and narrow, and sometimes slightly spatulate. Brown scarred areas were present on the petioles, the midveins, and on the knob-like central leaves. Leaves of some of the less-affected plants were showing quite severe distortions. Most of the damage was undoubtedly due to the external feeding of the nematodes, but some invasion of a few outer leaves had occurred. These leaves were a duller green in colour, and at this stage there was no interveinal chlorosis.

A number of these infested plants were planted out in the open ground at the end of May. Some did not survive but the others, even though the main shoots were killed, sent out new growth from the base. By the middle of June some normal endoparasitic damage with interveinal chlorosis was showing up on these plants. Later, in September, most of the leaf area had been invaded and killed by nematodes. The flower heads which had formed were small in size, and distorted, and were found to be heavily infested with nematodes living ectoparasitically.

As the aster seed had been sown in sterilized compost and the seedlings pricked out into the same medium, it was surmized that the infestation was probably seed-borne. Fortunately a supply of the same batch of seed was available for examination. Fourteen grammes of seed were placed in a Baermann funnel and over 300 nematodes emerged. It was decided to try to reproduce the damage in the laboratory on plants raised from this infested seed. Some of the seed was sown in sterilized soil in seed boxes, and when the seedlings reached the two true-leaf stage, fifty were removed for examination. These were placed in a Baermann funnel, and in the course of two weeks about 400 nematodes were collected. It is surprising that the nematodes emerged over such a long period, but it should be borne in mind that the seedlings remained green for a long time, and it is probable that the nematodes continued to feed and reproduce and only settled out when the conditions in the funnel became unfavourable to their continued existence. There were no obvious symptoms on these small seedlings. Later, about three weeks after pricking out the seedlings, typical symptoms developed on about 10% of the plants.

The seed had been grown on the nursery. The grower had selected for seed production some of the most vigorous plants from the main planting of asters just after flowering had commenced at the end of June in the previous year. These were replanted by themselves in the open ground in another part of the nursery. It is most unlikely that plants already infested were chosen, and it is probable that the plants were attacked in their new quarters. Evidence for this is provided by the fact that plants raised from another batch of seed obtained from plants remaining in the main bed of asters did not develop symptoms. In addition, the nursery was visited for another purpose in the previous year, and the damage in the main planting was very slight.

The method of threshing used by the grower was as follows. When ripe, the seed heads were collected, and threshed by rubbing between the hands and blowing out as much as possible of the débris. As would be expected a considerable amount of leaf and sepal débris remained mixed with the sample of seed. In the laboratory some of this débris was separated from the seed and soaked in water in a solid watch glass. Considerable numbers of nematodes emerged from this débris. However, nematodes also emerged in fairly large numbers from 100 seeds from which the débris had been removed, showing that the seed itself was infested. It was subsequently found

that the nematodes live an ectoparasitic existence in the flower heads, and hence can easily become attached to the seed when the flower dies, and the seeds ripen.

Seed-borne infestation in *A. ritzema-bosi* has not hitherto been recorded, although a preliminary note on this occurrence has been published (Brown, 1955). *A. ritzema-bosi* is most frequently recorded as a pest of chrysanthemums, and as this plant is propagated vegetatively seed-borne infestation is unlikely to occur.

The only other species in this genus known to be seed-borne is *A. oryzae*, the cause of white tip of rice. (Cralley and French, 1952.)

Southey (1952) has described symptoms on chrysanthemums caused by ectoparasitic attack, which are similar in many ways to those on the asters. The annual aster now joins the chrysanthemum as a plant upon which, under certain conditions, the chrysanthemum eelworm may live both ecto- and endoparasitically.

I wish to thank Mr. R. W. Rennison and Dr. Storey of the N.A.A.S. for bringing this problem to my attention and also Mr. W. E. Dant for the photographs.

References

Brown, E. B., 1955.—"A seed-borne nematode infestation in annual asters." *Nature, Lond.*, **175,** 178. (W.L. 14900)

Cralley, E. M. and French, R. G.—"Studies on the control of white tip of rice." *Phytopathology*, **42,** 6. (W.L. 16273)

Southey, J. F., 1952.—"Unusual chrysanthemum eelworm symptoms." *Plant Pathology*, **1,** 48–49.

Aster plants attacked by *A. ritzema-bosi*

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On *Ceylonocotyle scoliocoelium* (Fischoeder, 1904) and its Intermediate Host in Kenya, East Africa

By J. A. Dinnik

East African Veterinary Research Organization, Muguga, Kikuyu, Kenya

Amongst thousands of specimens of *Paramphistomum microbothrium* Fischoeder, *Cotylophoron cotylophorum* (Fischoeder), *Carmyerius exoporus* Maplestone, and *Carmyerius mancupatus* (Fischoeder) 645 specimens of *Ceylonocotyle scoliocoelium* (Fischoeder) were found in the rumen of one Zebu steer and in lesser numbers in the rumens of two other Zebu cattle slaughtered at the abattoir at Nakuru, Kenya. All three animals originated from a farm situated near Lake Ol Bolossat, at an altitude of about 7,700 ft. During the dry seasons of the year the cattle grazed in the vast swamps nearby. A survey of the swamps and the lake shores showed that only three species of snails, namely *Lymnaea caillaudi* Bgt., *Bulinus alluaudi* (Dautzenberg) and *Anisus natalensis* (Krauss) were present there. Specimens of *Anisus natalensis* collected from the places frequented by cattle emitted pigmented amphistome cercariae which formed cysts of two different sizes; the smaller cysts were from 0.24 to 0.27 mm. and the larger cysts from 0.31 to 0.35 mm. in diameter. Twenty-nine small and thirty-three large cysts were given to a goat and four mature specimens of *Ceylonocotyle scoliocoelium* and five specimens of *Carmyerius exoporus* were recovered from the rumen when the goat was slaughtered 130 days later.

Another sample of *Anisus natalensis* infested with larval stages of *Ceylonocotyle scoliocoelium* was collected by Dr. P. L. Le Roux from the swamp near Kisumu, Kenya and brought to our laboratory

for examination. Thirteen of the twenty-four snails emitted cercariae and altogether 474 encysted cercariae were given to a calf within a period of nineteen days. When the calf was slaughtered, 111 days after the first dose of the cysts was administered to it, thirty-six mature specimens of *Ceylonocotyle scoliocoelium* together with eighty-eight specimens of *Carmyerius exoporus*, and twenty specimens of *Carmyerius mancupatus* were recovered from the rumen.

The Morphology of the specimens of Ceylonocotyle scoliocoelium (Fischoeder, 1904) *found in Kenya*

The specimens recovered both from naturally infected cattle and from the calf infected experimentally are mature and measure from 2.7 to 4.0 mm. in length. The body of the species is broadly oval when viewed from the ventral side, reaching its maximum width at the level of the third quarter of its length. The ventral side of the body is flattened while the dorsal side is evenly curved. The acetabulum is situated subterminally with its aperture directed ventrally (Figs. 1 and 2).

The acetabulum is from 0.65 to 0.83 mm. in diameter and the ratio of its diameter to the body length varies from 1 : 4 to 1 : 5. The external circular musculature of the acetabulum, as seen in median sagittal sections, is weakly developed. Each half, dorsal and ventral, consists of sixteen to twenty muscle units which increase in size towards the inside. The internal circular musculature is more strongly developed, each half containing from twenty-four to thirty-four muscle units and the series extends 2–2½ times further than those of the external circular musculature.

The pharynx is from 0.37 to 0.50 mm. long ; the ratio of its length to the length of the body is from 1 : 8 to 1 : 10, and that of the length of the pharynx to the external diameter of the acetabulum is from 1 : 1.8 to 1 : 2.2. The internal circular muscle layer of the pharynx consists of small units. The internal longitudinal layer occupies about a quarter of the thickness of the pharynx wall and is indistinctly delimited externally. The external longitudinal layer is very narrow and the external circular layer is also weakly developed. The radial layer consists of thin sparsely distributed fibres running across the wall of the pharynx between the units of the external circular layer. The basal circular layer is present and composed of eight to ten muscle units. The lip sphincter, which is

only seen well in a lateral sagittal section of the pharynx as an arch-shaped muscular structure, exists on each side of the oral opening of the pharynx.

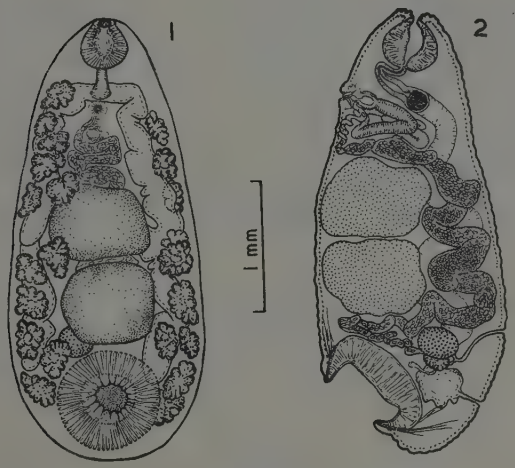

Fig. 1.—*Ceylonocotyle scoliocoelium* (Fischoeder) from experimentally infected calf, Kenya, E. Africa. Ventral view. Fig. 2.—*Ceylonocotyle scoliocoelium* (Fischoeder) from Zebu steer, Kenya, E. Africa. Sagittal section.

The oesophagus is from 0.50 to 0.72 mm. long, about one and a half times as long as the pharynx. In the anterior third of the oesophagus the two muscle layers are equally thin. The outer layer of longitudinal muscles remains thin throughout the length of the oesophagus. The muscle fibres of the inner layer, however, gradually increase in number in the posterior part of the oesophagus and form a strongly developed muscular bulb (Fig. 3). A group of circular muscle fibres forms a sphincter-like structure at the posterior end of the oesophageal bulb but it is not so strong and sharply delimited as the oesophageal sphincter in *C. streptocoelium* (Fischoeder, 1901). In the latter the muscular wall of the oesophagus is thickened only in the posterior half and the sphincter is seen as a separate structure at the end of the oesophagus (Fig. 4).

The gut caeca run from the end of the oesophagus straight towards the sides of the body where they turn, continue in wavy courses towards the posterior end of the body and terminate close to or at the level of the acetabulum.

The excretory bladder is roundish in outline and lies dorsal to the acetabulum. A short duct connects it with the excretory pore which is situated approximately on a level with the mid-part of the acetabulum, from 0.61 to 0.90 mm. from the posterior end of the body.

The testes are situated close to the ventral surface, one behind the other in the mid-line of the body. They are unlobed or only slightly lobed and approximately equal in size, measuring from 0.69 to 0.89 mm. dorso-ventrally and from 0.52 to 0.64 mm. antero-posteriorly.

The vesicula seminalis is long and coiled into several loops, while the pars musculosa is comparatively short, about 0.7–0.9 mm. long, and forms only one U-shaped loop. The pars prostatica is from 0.15 to 0.22 mm. long.

The ovary is oval to almost spherical in shape and lies between the posterior testis and the acetabulum, either on the right or left side in the dorsal part of the body. Mehlis' gland is situated close to the ovary. Measurements of the ovary are from 0.20×0.20 mm. to 0.33×0.41 mm. and those of Mehlis' gland from 0.13×0.15 mm. to 0.15×0.26 mm. A short oviduct connects the ovary with Mehlis' gland and is joined by Laurer's canal which runs dorsally and opens from 0.36 to 0.53 mm. anterior to the excretory pore.

Clusters of the vitelline gland, from nine to fifteen clusters each 0.2–0.3 mm. in size, extend from the level of the oesophagus to the middle part of the acetabulum on either side of the body.

The genital atrium is situated at the level of or slightly posterior to the junction of the oesophagus with the caeca. The genital sphincter is weakly developed and its circular muscles are not clearly delimited from the surrounding muscle fibres. The sphincter is, however, clearly recognizable as a distinct structure when it is contracted (Fig. 5). The radial musculature of the genital atrium is well developed and its fibres are evenly curved. The genital papilla varies in shape according to its degree of extension; the

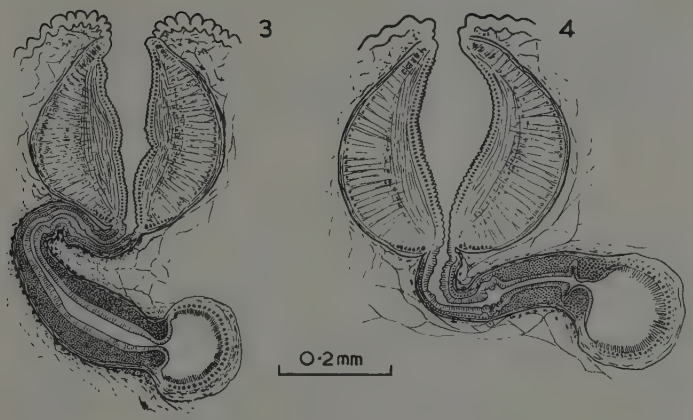

Fig. 3.—*Ceylonocotyle scoliocoelium* (Fischoeder) from Kenya, E. Africa. Pharynx and oesophagus in sagittal section. Fig. 4.—*Ceylonocotyle streptocoelium* (Fischoeder) from Queensland, Australia. Pharynx and oesophagus in sagittal section.

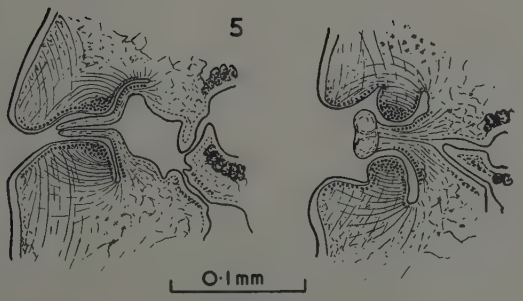

Fig. 5.—*Ceylonocotyle scoliocoelium* (Fischoeder) from Kenya, E. Africa. Genita atrium.

sphincter of the papilla is faint but quite recognizable as a layer of circular muscle units lying at the base.

The eggs are 0.137–0.150 mm. × 0.069 – 0.075 mm. when measured fresh ; the measurements are slightly less in specimens embedded in Canada balsam.

Discussion

Fischoeder (1904) described the species *Paramphistomum scoliocoelium* from specimens recovered from the rumen and reticulum of *Buffelus indicus* and *Bos taurus* in Indo-China. This species, together with four other species, was suppressed by Maplestone (1925) as a synonym of *Paramphistomum orthocoelium* Fischoeder, 1901, and this view was accepted by Fukui (1929), Travassos (1934) and Dawes (1936). However, Nasmark (1937), by sectioning and examining specimens from the original material has confirmed Fischoeder's description and the validity of the species. A new genus, *Ceylonocotyle* Näsmark, 1937, was proposed to include the following four species in which Laurer's canal opens anterior to and does not cross the excretory canal.

Species	Host and Geographical locality
C. orthocoelium (Fischoeder, 1901)	—*Bubalus bubalis*, Ceylon
C. dicranocoelium (Fischoeder, 1901)	—*Bubalus bubalis*, Hue, Indo-China
C. streptocoelium (Fischoeder, 1901)	—*Bubalus bubalis*, Ceylon
C. scolioceolium (Fischoeder, 1904)	—*Bubalus bubalis*, Saigon, Indo-China
	Na-Trang, Indo-China
	Punjab, India
	—*Bos taurus*, Na-Trang, Indo-China
	Calcutta, India

A hundred mature specimens from the rumen of a Zebu bull and two mature specimens from a goat collected in Malaya were referred by Dawes (1936) to *Paramphistomum orthocoelium* Fischoeder, 1901, although he experienced some difficulty in identifying the specimens from the goat. Of the forms regarded by Maplestone as synonyms of *P. orthocoelium* they most closely resembled Fischoeder's *P. streptocoelium*.

Recently *Ceylonocotyle streptocoelium* (Fischoeder) has been found outside South and South-East Asia in cattle (*Bos taurus*) in Queensland, Australia, and its life history has been studied in detail by Durie (1951 and 1953). An intermediate host of the species was found to be a small planorbid snail, *Glyptanisus gilberti* Dunker.

The morphology of the specimens collected in Kenya, East Africa, agrees closely with the original description and illustrations of *C. scoliocoelium* (Fischoeder, 1904) as well as with the re-description of the species as given by Näsmark (1937). The presence of a long oesophageal bulb, which is clearly distinct from the oesophageal sphincter of *C. streptocoelium* (Fischoeder, 1901), a lip sphincter of the pharynx and a genital sphincter enable us to refer the specimens found in cattle in Kenya to *Ceylonocotyle scoliocoelium* (Fischoeder, 1904). The dimensions of these specimens differ slightly from those given in the original description of the species. Although Fischoeder's material included mature specimens of the species from 2.0 to 6.5 mm. long, in the description of the species he only quoted the measurements of specimens from 5.0 to 6.0 mm. long. Our material, collected from both naturally and experimentally infected cattle, consists of mature flukes varying in length from 2.7 to 4.0 mm. As the flukes from the experimentally infected calf cannot be more than 111 days old, it appears that all our material consists of young mature specimens of the species and, therefore, the measurements of the various organs in them are proportionally smaller than those of the bigger, and presumably older, specimens which were measured by Fischoeder.

SUMMARY

1. *Ceylonocotyle scoliocoelium* (Fischoeder, 1904) is recorded from cattle in Kenya, East Africa, and a description of the specimens is given.

2. An intermediate host was found to be a small planorbid snail, *Anisus natalensis* (Krauss).

REFERENCES

DAWES, B., 1936.—"On a collection of *Paramphistomidae* from Malaya, with revision of the genera *Paramphistomum* Fischoeder, 1901, and *Gastrothylax* Poirier, 1883." *Parasitology*, **28**, 330–354. (W.L. 16035.)

Durie, P. H., 1951.—"The paramphistomes (Trematoda) of Australian ruminants. Part I. Systematics." *Proc. Linn. Soc. N.S.W.*, **76,** 41–48. (W.L.16790a.)

———1953.—"The paramphistomes (Trematoda) of Australian ruminants. Part II. The life history of *Ceylonocotyle streptocoelium* (Fischoeder) Nasmark and of *Paramphistomum ichikawai* Fukui." *Aust. J. Zool.*, **1,** 193–222.

Fischoeder, F., 1901.—"Die Paramphistomiden der Saugetiere." *Zool. Anz.*, **24,** 365–375. (W.L. 23833.)

———1904.—"Beschreibung dreier Paramphistomiden-Arten aus Saugethieren." *Zool. Jber.* (Syst.) **20,** 453–470. (W.L. 23835.)

Fukui, T., 1929.—"Studies on Japanese Amphistomatous parasites, with revision of the group." *Jap. J. Zool.* **2,** 219–351. (W.L. 10881w.)

Maplestone, P. A., 1923.—"A revision of the amphistomata of mammals." *Ann. trop. Med. Parasit.*, **27,** 113–212. (W.L. 1063.)

Näsmark, K. E., 1937.—"A revision of the trematode family *Paramphistomidae.*' *Zool. Bidr. Uppsala*, **16,** 301–565. (W.L. 23836.)

Travassos, L., 1934.—"Synopse dos Paramphistomoidea." *Mem. Inst. Oswaldo Cruz*, **29,** 19–178. (W.L. 13465.)

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Seasonal Variations in the Hatching Responses of the Potato-root Eelworm, *Heterodera rostochiensis* Wollenweber, and Related Species

By R. D. Winslow*
School of Agriculture, University of Cambridge

Several workers have investigated seasonal variation in the emergence of larvae from cysts of the potato-root eelworm (*Heterodera rostochiensis* Woll.) with apparently conflicting results. It is generally agreed that emergence from cysts freshly removed from the field is greatly diminished in the winter months (Triffitt, 1930), but Triffitt also found that cysts taken from soil in the growing season and stored air-dry failed to respond to root diffusate in winter. As Ellenby (1955) has suggested, this apparent seasonal decline in hatching may have been merely a reflection of seasonal decline in the potency of the root diffusate used, which was collected freshly from growing plants. This, however, could not explain the findings of Calam, Raistrick and Todd (1949), that marked seasonal variation occurred in the hatchability of cysts collected in August and stored at 23°C. and 50% humidity, even when a standardized stimulant was used in hatching tests conducted at intervals throughout the year. Feldmesser and Fassuliotis (1950) and Lownsbery (1951) claimed that there were seasonal differences in the hatchability of the "golden nematode" on Long Island. Lownsbery found greatly decreased hatching in December from a cysts-debris mixture collected in July and stored air-dry at 25°C. Fenwick and Reid (1953) claimed that no seasonal decrease in hatch occurred from cysts stored air-dry at room temperature provided they were collected in spring or summer, and similar findings were reported by Ellenby (1955), using a cysts-debris mixture collected in September and stored air-dry at 23°C., 5°C. and room temperature. Thus there appear to be two schools of though, the one claiming that there is a winter dormancy not directly dependent on temperature (Lownsbery); the other that there is no true dormancy, the decreased hatching in

* Present address: Science Service Laboratory, London, Ontario, Canada.

winter being merely a product of the environmental conditions (Fenwick and Reid; Ellenby). The experiments described below were designed to throw some light on the problem and to extend the investigations to other species of *Heterodera*.

Experiments

In preliminary tests carried out in 1952–53, field plots infested with beet and potato-root eelworms (*H. schachtii* Schmidt and *H. rostochiensis* Woll. respectively) were sampled at intervals over a twelve-month period. On each occasion the soil samples were allowed to dry in the laboratory atmosphere for seven days. Cysts recovered from these samples (by flotation and sieving) were incubated in stimulant root diffusate for twenty-one days at 25°C. At the end of each week, the diffusate containing the hatched larvae was drawn off and more diffusate added from the stock bottle stored at 1°C. Under these conditions beet eelworm hatched readily throughout the winter months, when very few larvae emerged from the potato eelworm cysts.

In 1954–55 a similar experiment was conducted, involving seven species of *Heterodera*. In this case the soil samples were allowed to dry for three days in the laboratory before the cysts were recovered for the hatching tests. The results are given in Fig 1.

Concurrently with this experiment, another was carried out to examine the effects of various soil storage temperatures on the hatchability of three eelworm species. In each case soil was taken on 29th August 1953 from an infested plot on which a host crop had been grown in 1953 but not in 1954. The soil was thoroughly mixed while still reasonably moist and divided into eight lots, five of these being stored at constant temperatures, in closed, but not air-tight containers. The remaining three were stored at fluctuating temperatures. At monthly intervals each lot was sampled, the soil samples being allowed to dry for two days in the laboratory atmosphere. Cysts recovered from the air-dry samples by flotation and sieving were put directly into stimulant root diffusate held at 25°C. and the numbers of larvae hatching over a period of twenty-one days estimated. Throughout the experiment, the root diffusate used for each eelworm species was drawn from the same stock bottle stored at 1°C. The experiment had to be terminated after eight months, owing to the writer's departure from Cambridge. The results are presented graphically in Fig. 2.

FIG. I

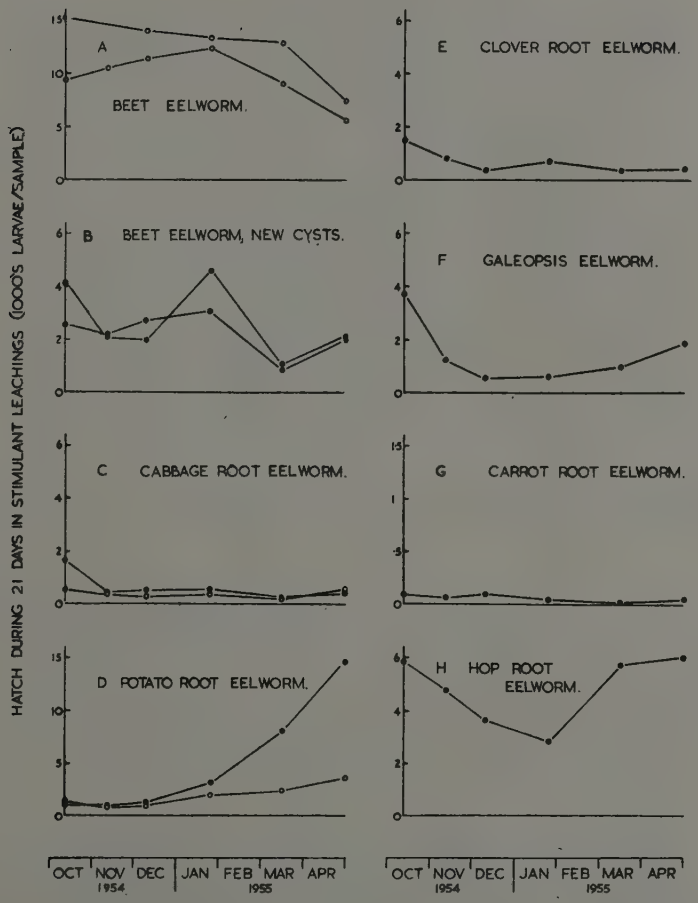

Fig. 1. Seasonal variations in larval emergence from cysts of seven species of *Heterodera*. Soil samples were taken from infested plots throughout the winter of 1954–55 and the cysts recovered from these samples incubated in stimulant root leachings.

Key : —— o —— =plots was fallow or carried non-host in 1954.
—— ● —— =plot carried a host-crop in 1954.

Results

Judging from Fig. 1, A, there was no seasonal decline in the hatchability of beet eelworm populations containing no new (=1954) cysts. The decreased hatch at the end of April was due to loss of contents from the cysts, presumably by spontaneous hatching in the soil in the spring. *In vitro* tests have shown that the spontaneous or water hatch of some beet eelworm populations may be more than 50% of the stimulated hatch (Winslow, 1955). Hatching from populations containing new cysts was more variable (Fig. 1, B) and here there may be some seasonal decrease in hatching. Hatching from cysts of cabbage eelworm (*H. cruciferae* Franklin), potato eelworm and carrot eelworm (*H. carotae* Jones) in this (Fig. 1, C, D and G) and all other experiments conducted by the writer showed a marked seasonal decline, larval emergence in the winter being almost negligible, usually less than 5% of the total egg content of the sample. Potato eelworm apparently entered this dormancy earlier in the autumn and emerged from it earlier in the spring than did cabbage eelworm. Clover, *Galeopsis* and hop eelworms [(*H. trifolii* (Goffart), *galeopsidis* (Goffart) and *humili* Filipjev respectively)] showed partial dormancy in this experiment (Fig. 1, E, F and H) but these populations contained some newly-formed (1954) cysts, and newly-formed cysts appear to be less hatchable in winter than older cysts.

From Fig. 2 it is clear that when the soil was removed from the field, potato eelworm had started to enter the dormancy period and by the end of October hatching was virtually nil in all treatments. Storage at the higher temperatures, notably 25°C., shortened the period of dormancy. By the end of April hatching was uniformly high in all treatments ; thus the organism, having started to enter the dormancy phase before the commencement of the experiment, completed the cycle irrespective of storage temperature.

Cabbage eelworm behaved initially in a similar manner, all samples becoming dormant in winter. By the end of the experiment, high hatching was occurring from cysts from the soil stored at 25°C., suggesting a pattern of behaviour like that of potato eelworm but later in the season. This bears out the results shown in Fig. 1.

In the case of beet eelworm, winter dormancy was much less pronounced. In general there was a steady fall in numbers of larvae hatching, probably due in part to loss of viability from parasitism,

FIG. II

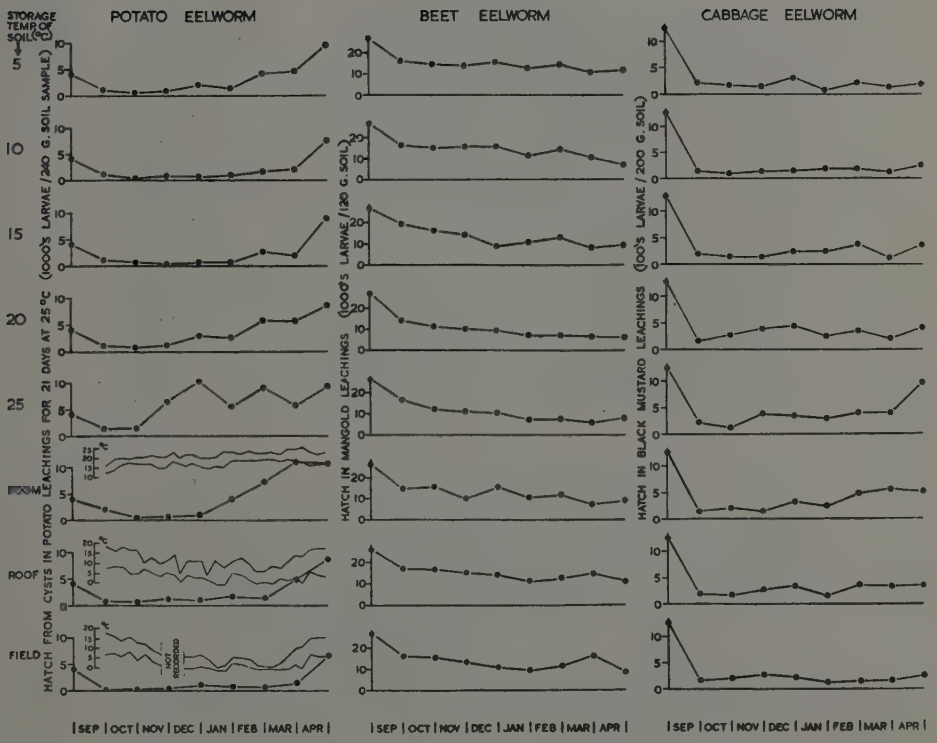

Fig. 2. Effect of storing infested soils at various temperatures on the subsequent hatchability of three species of *Heterodera*. In the case of those treatments in which soil storage temperature was not constant, weekly maximal and minimal temperatures are indicated for the soil infested with potato eelworm. Similar temperatures were recorded for the other two soils.

exhaustion of food reserves and other causes, and in part to spontaneous hatching in the soil, since the fall was greater in the higher temperature treatments.

Discussion

The apparent contradictions in the findings of various workers investigating seasonal variation in hatching of potato eelworm are almost certainly due to differences in experimental conditions. Climatic differences might explain why the American workers, Feldmesser & Fassuliotis and Lownsbery obtained results at variance with those of the British workers, Fenwick & Reid and Ellenby. It is more difficult to reconcile the findings of these latter authors with those of Calam, Raistrick & Todd, but Calam *et alia* maintained the cysts at constant humidity (50%) while those of Fenwick & Reid and Ellenby were stored air-dry, i.e. at fluctuating humidity. In the experiments described in this paper the cysts were stored in moist soil, which was only allowed to become air-dry just before cyst recovery. This was done by flotation in water, passing the float through the usual two sieves and collecting the intermediate fraction on filter paper from which the cysts were removed while still moist. At no time did the cysts become air-dry.

Fenwick & Reid concluded that the seasonal fluctuations in hatch described by previous workers were the effect of environmental conditions, and Ellenby felt that the dormancy was probably not fundamental to the nature of the animal. But air-drying of cysts is rather drastic treatment and the hatching behaviour of eelworms stored under more natural conditions, i.e. as cysts in soil, may give a better indication of the true nature of the animal in this respect. It is unfortunate that the experiment described above (Fig. 2) did not start before the onset of winter dormancy. Had infested soil been collected from the field earlier in summer and stored at one of the higher temperatures (25°C., 20°C., or room temperature) the eelworm might not have entered the dormancy phase. On the other hand the perpetuation of near-winter temperature did not prolong the dormancy phase in spring since by the end of April high hatching was occurring from cysts recovered from the soil stored at 50°C. This suggests that the dormancy of potato-root eelworm does not depend simply on environmental conditions and supports the conclusion of Lownsbery, that the temperature is not the factor directly governing the seasonal behaviour. That storage of soil at high temperature could shorten the dormancy period, however, shows that dormancy is not altogether temperature-independent.

The writer concludes that there is a winter dormancy in the hatchability of some species of *Heterodera*. This dormancy varies in degree with eelworm species, being slight or negligible in the case of beet eelworm and more pronounced in the case of potato-root eelworm. The nature of the dormancy is not fully understood but it does not appear to be wholly dependent on immediate environmental conditions ; it is something more complex, possibly induced in some way by previous environmental conditions. Once commenced upon, the dormancy can be shortened by storing the infested soil at high temperature, but the cessation of dormancy in the spring is not necessarily associated with rise in soil temperatures.

SUMMARY

There is a winter dormancy in the hatchability of some species of *Heterodera*. The dormancy varies in degrees with eelworm species, being slight in the case of beet eelworm and more pronounced in the case of potato-root eelworm and some other species. It is not wholly dependent on immediate environmental conditions but possibly is induced by previous conditions. The dormancy can be shortened by storing infested soil at certain temperatures, but the cessation of dormancy in spring is not necessarily associated with rise in soil temperature.

ACKNOWLEDGMENTS

I wish to thank my colleagues in the School of Agriculture, Cambridge for assisting in the work, especially Mr. F. G. W. Jones for his interest and advice, and Messrs. R. Day and P. Halfpenny for technical assistance. The work was financed by the Sugar Beet Research and Education Committee.

REFERENCES

CALAM, C. T., RAISTRICK, H. and TODD, A. R., 1949.—"The potato eelworm hatching factor. 1. The preparation of concentrates of the hatching factor and a method of bioassay." *Biochem. J.*, **45**, 513. (W.L. 2967)

ELLENBY, C., 1955.—"The seasonal response of the potato-root eelworm *Heterodera rostochiensis* Wollenweber ; emergence of larvae throughout the year from cysts exposed to different temperature cycles." *Ann. appl. Biol.*, **43**, 1. (W.L. 1025)

FELDMESSER, J. and FASSULIOTIS, G., 1950.—"Reactions of the golden nematode of potatoes, *Heterodera rostochiensis* Wollenweber, to controlled temperatures and to attempted control measures." *J. Wash. Acad. Sci.*, **40**, 355. (W.L. 11600)

Fenwick, D. W. and Reid, E., 1953.—"Seasonal fluctuations in the degree of hatching from cysts of the potato root eelworm." *Nature, Lond.*, **171**, 47. (W.L. 14900)

Lownsberry, B. F., 1951.—"Larval migration from cysts of the golden nematode of potatoes, *Heterodera rostochiensis* Wollenweber." *Phytopathology*, **41**, 889. (W.L. 16273)

Triffitt, M. J., 1930.—"On the bionomics of *Heterodera schachtii* on potatoes, with special reference to the influence of mustard on the escape of larvae from the cysts." *J. Helminth*, **8**, 19. (W.L. 11224c)

Winslow, R. D., 1955.—"The hatching responses of some root eelworms of the genus *Heterodera*." *Ann. appl. Biol.*, **43**, 19. (W.L. 1025)

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A New Technique for Counting Eggs of *Fasciola hepatica* in Cattle Faeces

By W. Dorsman
State Serum Institute, Rotterdam

The principle of separating fluke eggs from faeces by sieving, which is not new (Willmott and Pester, 1952), is here applied to develop an egg-counting method, the essential features of which are a special technique to transfer the eggs quantitatively to a measured small amount of water and a new technique of filling an egg-counting slide.

Description of The Method

After a weighed quantity of faeces is thoroughly mixed with a measured amount of water, a sample is drawn corresponding to about 3 grammes faeces. The sample is washed with water on a sieve (Fig. 1,a) with meshes of about 0.16 mm., of an empty Baermann apparatus.

With a small spray attached to a tap the faecal suspension is washed through the screen (which retains the coarse particles) until the water level reaches the sieve. The sieved and diluted faecal suspension is subsequently passed through a second sieve (c), consisting of a metal tube in which copper gauze with meshes of about 0.05 mm. is inserted. A piece of a flexible, transparent plastic tube (d) is attached to the upper end of the metal tube, thus forming an extension of sieve (c), which prevents fluid from splashing away. The plastic tubing is provided with a hole, about 3 mm. in diameter, which is kept closed by a piece of bicycle tube (e) around the plastic tube. The fluid is forced through sieve (c) from the funnel in short abrupt jets, effected by intermittently releasing the pinchcock (b). In this way the narrow meshes of the gauze are prevented from being blocked by flocky material.

When the funnel is empty, the washing through sieve (a) and filtering through sieve (c) are repeated twice in the same way.

Since none of the eggs of *Fasciola hepatica* passes through the fine gauze, all those which were present in the faecal sample are collected in sieve (c), freed from coarse particles as well as from

flocky material and colouring-matter. Any material which has splashed on the walls of the plastic and the metal tube is now washed to the gauze with a fine jet of water. A few drops of a methylene blue solution are now distributed over the surface of the gauze in order to stain the debris. The yellow-brown colour of the fluke eggs remains unchanged, so that they are more readily distinguished in the counting slide. The excess of stain is washed away by again passing water through sieve (c) from the funnel, sieve (a) having now been removed. In this operation the filling of the funnel is now accomplished by a strong jet of water against the wall, so that whirling of the water results, in order to loosen eggs which may be still attached to the funnel wall.

The material is again washed quantitatively to the gauze and a segment of the gauze surface under the small hole in the wall of the plastic cylinder is freed from particles by transferring them in horizontal direction over the gauze with the same fine jet of water. The part (f), which will become the bottom, is now inserted in the free end of the plastic cylinder, the sieving apparatus being still kept in the same perpendicular position (Fig. 2).

When (f) has been inserted the apparatus is turned upside down, and 24 cc. of water is carefully poured on the gauze (Fig. 3). Since the narrow hole in the plastic tubing is still closed by the strap (e), the air inside cannot escape and the water remains on the gauze. The apparatus is then held in both hands and the rubber strap is partially rolled up with both thumbs to uncover the hole in the plastic tube. This allows the water to fall through the gauze, taking with it the entire sediment which was resting against the underside of the gauze to the bottom of the vessel. It is advisable to keep the apparatus in a slightly oblique position so that while the hole is uncovered this is not wetted by the falling water.

The sieve is now taken off the plastic tube, a small iron rod is put in the fluid and the vessel is placed on a magnetic stirring apparatus. During the stirring 6 cc. of a freshly shaken 2.2% solution of carboxymethylcellulose ("CellofasB" : I.C.I.) is gradually poured in the vessel, after which the lid (g) is attached to the plastic tube to close the vessel (Fig. 4).

The lid is provided with two holes through one of which air can be forced into the vessel by means of a rubber bulb (h). The other hole is closed with a small rubber stopper, which is perforated to hold a narrow rigid tube (k) to which a length of rubber tubing (l) is attached.

The suspension is stirred long enough to ensure even distribution of the eggs, the length of the period of stirring depending on the size and shape of the stirrer and on the rotational speed. A counting slide, similar to the McMaster slide, is now filled as quickly as possible by squeezing the bulb, thus causing fluid to be discharged from the tubing (l). During this operation the stirring is continued. If the magnetic stirring apparatus is placed close to the microscope it is possible to fill the counting slide lying on the stage of the microscope so that it does not need to be replaced after filling.

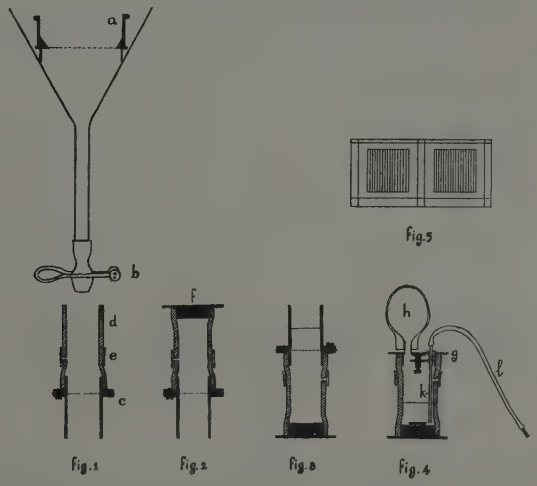

fig.1 fig.2 fig.3 fig.4 fig.5

The eggs become evenly distributed within the counting chamber before sinking to the bottom since their sedimentation rate is slowed down by the carboxymethylcellulose. They are counted under a low power microscope, preferably a dissecting microscope with a magnification of about 40 x, within a square marked on the bottom of the counting chamber. As the square contains the eggs which originate from 1 cc. of the suspension, their number, multiplied by ten, corresponds to the number of *Fasciola* eggs per gram faeces.

The whole procedure, from the moment of putting the faecal sample on the sieve in the funnel, and including counting the eggs in both squares of the counting slide, takes about a quarter of an hour.

Testing of The Method

As the correctness of the counts obtained with the method is not proved by their reproducibility alone, the method was checked by adding a known number of eggs to a faecal sample.

Material and methods

The anterior portions of fresh adult flukes (*Fasciola hepatica*), containing the uterus, were cut to small pieces with scissors. The material was washed with tap water on a sieve with meshes of about 0.21 mm. The eggs passed through the sieve, freed from tissue particles, were concentrated by centrifugation. Eggs of normal appearance could easily be distinguished from damaged ones, which were distorted or had lost part of their contents.

A drop of the egg suspension was put on a slide (provided with parallel lines to facilitate systematic searching under the microscope) and was covered with a coverslip. After all the eggs of normal appearance in the preparation had been counted, the coverslip was carefully lifted by means of forceps, and the eggs were washed from both slide and coverslip with part of a measured quantity of water into a faecal suspension made up from known amounts of faeces and water. When after several drops had been counted the final number of eggs needed to enrich the faecal sample had been computed the residue of the measured quantity of water was added to the faecal suspension.

Results

1. A weighed quantity of cow faeces of normal consistency, to which was added an equal quantity of water, was thoroughly mixed by a small screw-propeller coupled to a common laboratory stirrer (faecal suspension A).

The number of *Fasciola* eggs in a sample of 6 cc., containing approximately 3 gm. faeces, was determined by the method described above. After the two compartments of the slide were filled and the eggs in both counted, the contents were poured back into the stirring vessel. This was repeated twice.

Number of eggs in counting slide		mean egg counts
8	9	85 e.p.g.*
8	10	90 e.p.g.
8	8	80 e.p.g.
		mean : 85 e.p.g.

2. To 18 cc. of the faecal suspension (A) were added 147 eggs of *Fasciola hepatica* together with 18 cc. water. Whilst the enriched suspension was stirred with a spoon small quantities were taken out to a total volume of 12 cc., corresponding to about 3 gm. faeces, in which the number of eggs was determined as under 1.

Numbers of eggs in slide		mean egg counts
10	10	100 e.p.g.
11	10	105 e.p.g.
11	10	105 e.p.g.

mean determined enrichment : 103—85= 18 e.p.g.

real enrichment : $\frac{147}{9}$ = **16 e.p.g.**

3. Procedure as under 2, but with the addition of 398 eggs.

Numbers of eggs in slide		mean egg counts
13	14	135 e.p.g.
13	14	135 e.p.g.
13	13	130 e.p.g.

mean determined enrichment : 133—85= 48 e.p.g.

real enrichment : $\frac{398}{9}$ = **44 e.p.g.**

4. Procedure as under 2, but with the addition of 843 eggs.

Numbers of eggs in slide		mean egg counts
18	17	175 e.p.g.
18	18	180 e.p.g.
18	18	180 e.p.g.

mean determined enrichment : 178—85= 93 e.p.g.

real enrichment : $\frac{843}{9}$ = **94 e.p.g.**

The results of these experiments demonstrate that all eggs are recovered and that no constant percentage is lost.

* e.p.g. = eggs per gram faeces.

TECHNICAL DETAILS

The sieve (Figs. 1 to 3 : c) can be manufactured from any metal, preferably stainless. The experimental apparatus, made in this Institute, consists of two pieces of brass pipe, so called condenser pipe, 20 mm. and 55 mm. long respectively, with inner diameter of

38 mm. and outer diameter of 42 mm., round one of the ends of each of which is screwed a flange of about 5 mm. thickness. Between both flanges, which can be pressed against each other by means of three screws, the copper gauze with meshes of about 0.05 mm. is mounted in the following way.

A piece of the gauze of the same diameter as the flanges and a ring of polyethylene of the same shape as the flanges but about 2 mm. thick are put between the two flanges after having both gauze and ring provided with three holes for the screws. After the screws are tightened, the flanges are gently heated with a Bunsen burner until the plastic ring becomes soft. As soon as this occurs the screws are carefully tightened further, taking care that the plastic does not protrude over the surface of the gauze inside the pipes. In this way the plastic is forced through the meshes of the gauze between the two flanges, so that the gauze becomes enclosed in a thin plastic layer, which makes a perfectly air- and water-tight joint between the two flanges. After cooling, the gauze enclosed in the plastic ring can easily be taken out if desired by unscrewing the apparatus. To avoid a groove between the inner surface of the plastic tubing and the outer surface of the shorter pipe, to which the tubing is attached, the outer edge of the pipe should not be rounded.

The diameter of the plastic tubing of about 100 mm. length (flexible transparent polyvinyl) is at the inside 40 mm. and at the outside 50 mm. The inner edges are somewhat rounded by scraping with a knife to facilitate introduction of the sieve, the bottom or the lid. To make them fit better one of these is attached to the plastic tubing, after which they are dipped in hot water for a short time.

The hole with a diameter of about 3 mm. has been bored at about 23 mm. from one end of the plastic tubing. This end, nearest to the hole, is attached to the sieve, which later in the procedure is replaced by the lid.

The bottom can be made of any rigid antimagnetic material. The difference in thickness between the central part and the projecting rim should preferably be more than 5 mm.; in our model it is 12 mm. On the other hand the total thickness of the bottom should not be so great as to interfere with proper functioning of magnetic stirring. Thus the maximum thickness should be adapted to the magnetic power of the stirring apparatus to be used. The

edge of the central part should not be rounded. The diameter of the thick central part of the bottom is made equal to the outer diameter of the metal pipes of the sieve.

The cylindrical part of the lid, about 15 mm. long, which also is attachable to the plastic tubing, is easily made from the same pipe which is used for the sieve. The hole, which is closed with the small rubber stopper, should be bored as far from the centre of the lid as possible, so that the tube (k : Fig. 4) is parallel to the wall of the plastic tubing, without touching it, when the vessel is closed. Since the position of the tube (k) is insufficiently fixed by the rubber stopper alone, a second point of support, consisting of a short tube which just fits round tube (k) and which can be adjusted in the position wanted, is made inside the lid (Fig. 4). It is of advantage if the tube (k), like the plastic tubing, is transparent to enable one to see whether it is filled with fluid or not. The author has found celluloid tube with outer diameter of 3.5 mm. and inner diameter of 2.5 mm. suitable for the purpose, since it is somewhat flexible and can be forced in the direction wanted.

The actual stirrer used in this laboratory is an iron cylinder, 4 mm. in diameter and 25 mm. long.

The counting slide used (Fig. 5) was made of acrylic sheet ("Perspex", I.C.I.), 1.6mm. thick. Two slides, 85×42mm. and 85×35mm. respectively are separated by three pieces of 42×5 mm. in such a way that a small platform of 5 mm. on the broadest bottom slide remains uncovered to make filling possible. Before mounting the parts by means of plastic glue, a square of 25×25 mm., divided by parallel lines to facilitate systematic searching under the microscope, is scratched in the surface of the bottom slide in the centre of the two squares of 35×35 mm. which form the future bottoms of the two counting chambers.

The counting slide permits observation of the eggs under a 16 mm. objective.

Additional directions to obtain optimum results

1. The viscosity of a carboxymethylcellulose solution diminishes with rise of the temperature. With excessive high room temperatures it may be necessary to use a greater amount or a higher concentration than proposed.

2. The rotational speed of the magnetic stirring apparatus should not be adjusted too high. When the stirring rod is stopped it should immediately resume its movement when released. Another risk of too high a speed, which should be avoided, is the drifting away of the stirring rod from the centre of the bottom, which can be caused by the resistance to the circular movement of the fluid brought about by the tube (k). Also the formation of many air bubbles in the fluid will be avoided by stirring not too fast.

3. Whilst closing the vessel with the lid compression of air is to be avoided.

4. During stirring before filling the counting slide the tube (k) should be completely filled with air. This is accomplished by successively uncovering the hole in the plastic tubing, compressing the rubber bulb, closing the hole and allowing the bulb to expand.

5. When the first compartment of the counting slide has been completely filled in the shortest possible time, the end of the tube (l) is taken away from the platform of the slide before allowing the bulb to expand again. Since the air sucked back into the vessel forms temporary air bubbles in the fluid, half a minute should elapse before filling the second compartment.

Summary

The essential features of the method, which is described in detail, are a sieving technique to collect quantitatively the *Fasciola hepatica* eggs of a sample of cattle faeces, a new technique for transferring quantitatively the eggs to a measured amount of water, and a new technique for obtaining even distribution of the eggs within a counting chamber. Technical details for manufacturing the apparatus and the counting slide, designed to perform the different stages of the method, are given. The correctness of the resulting counts is demonstrated.

Acknowledgment

I am greatly indebted to Mr. and Mrs. Neale Dowswell who were so kind as to correct the manuscript.

Reference

Willmott, S., and Pester, F. R. N., 1952.—"Variations in faecal egg-counts in Paramphistome infections as determined by a new technique.' *J. Helminth.*, **26**, 147–156. (W.L. 11224c)

Journal of Helminthology, Vol. XXX, *Nos.* 2/3, 1956, *pp.* 178–182.

The First Record of *Raillietina (Raillietina) celebensis* (Janicki, 1902), *(Cestoda)* in Man from Australia, with a Critical Survey of Previous Cases

By Jean G. Baer and Dorothea F. Sandars*
University of Neuchâtel

Amongst material sent by Dr. M. J. Mackerras of the Queensland Institute of Medical Research, Brisbane, to one of us for identification were (a) a number of gravid proglottides collected from the faeces of a twenty-month old child from Brisbane, Australia, and (b) tapeworms from the duodenum of rats identified as *Rattus assimilis* Gould, from Mt. Glorious, South Queensland. They were all collected in 1955.

Although only ripe proglottides were recovered from the child, these have been identified as *Raillietina* (*Raillietina*) *celebensis* (Janicki, 1902) on the basis of the position of the genital pore which is, in each proglottid, close to the anterior border. The cirrus pouch is 137 to 160μ long and 46 to 69μ in diameter. Each egg-capsule contains 1 to 4 eggs, 34 to 46μ in diameter. The proglottides have a markedly torulose appearance and are 1 to 2 mm. long and 0.75 to 1.2 mm. wide (Fig. B).

Among the cestodes from rats, were several complete worms identified as *Raillietina* (*R.*) *celebensis* (Janicki, 1902). These are 35 to 175 mm. in length, with a maximum width of 1.4 to 1.75 mm. The scolex, which is 274 to 411μ long and 480 to 803μ in diameter, bears four suckers each 114 to 183μ in diameter and with a number of very minute spines on the inside walls. The rostellum is 105 to 123μ in diameter and bears 160 hooks 18 to 23μ long arranged in two circlets. The mature proglottides have the typical anatomy

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for this genus. The cirrus pouch is 114 to 151μ long and 50 to 73μ in diameter and the genital pore is always within the anterior quarter of the proglottid length. There are 28 to 30 testes (7 to 9 poral ; 21 aporal) which have a diameter of 37 to 46μ. In gravid proglottides there are 100 to 140 egg-capsules and in each capsule 1 to 4 eggs of 27μ diameter. The nearer to the posterior end of the worm the gravid proglottides are, the more torulose they are in shape (Fig. A).

These tapeworms fall within the incomplete description given by Janicki (1902), for *Davainea celebensis* and the redescription given by Meggitt & Subramanian (1927). There have been described no less than six further species of *Raillietina* (*Raillietina*) which have been, or may be, regarded as synonyms of *R.* (*R.*) *celebensis*.

Joyeux & Baer (1929) recognised *R.* (*R.*) *formosana* (Akashi, 1916) as a synonym. An examination of Table I will show that the variety *R.* (*R.*) *celebensis paucicapsulata* Meggitt & Subramanian, 1927, *R.* (*R.*) *funebris* Meggitt & Subramanian, 1927, *R.* (*R.*) *garrisoni* Tubangui, 1931, *R.* (*R.*) *sinensis* Hsu, 1935, all have the same range in numbers and size of rostellar hooks and testicular follicles and agree in the anterior position of the genital pore. They also agree in other features, such as in the presence of spines both on the rostellum and the suckers and in the range for number of eggs in each capsule. *R.* (*R.*) *murium* Joyeux & Baer, 1936, when considered from the description given, is strikingly different from the other forms in the number of rostellar hooks and in the number of testicular follicles. However, re-examination of the material described by these authors reveals that the number of hooks and testes do fit within the range for *R.* (*R.*) *celebensis*. All these species are from the same geographic region. They are all now, regarded as synonyms of *Raillietina* (*R.*) *celebensis* (Janicki, 1902).

Material from *Rattus norvegicus* from Hanoi has also been examined and its features shown in Table I. It is identified as *R.* (*R.*) *celebensis*.

The ripe proglottides from the child bear a striking resemblance to the gravid proglottides of *R.* (*R.*) *celebensis* from the Queensland rat. They may be regarded as being from the same locality, since Mt. Glorious is within 30 miles from the centre of Brisbane. There can be little doubt that they are infections with the same species of cestode and that the child has acquired, secondarily, this infection from normal rat hosts.

Table I

	R. (*R.*) *celebensis* present paper	*R.* (*R.*) *celebensis* present paper	*R.* (*R.*) *celebensis* var. *paucicapsulata* Meggitt & Subramanian, 1927	*R.* (*R.*) *funebris* Meggitt & Subramanian, 1927	*R.* (*R.*) *garrisoni* Tubangui, 1931	*R.* (*R.*) *sinensis* Hsu, 1935	*R.* (*R.*) *murium* Joyeux & Baer, 1936
Length in mm.	35–175	60–110	242	32	600	120	16–35 (40)
Width in mm.	1.4–1.75	1.5–2.5	1.04	0.76	1.4–1.65	0.87	1 (1.4)
Number of rostellar hooks	160	90–100	100–120	80–100	90–140	120	240–250 (100–120)
Size of rostellar hooks in μ	18–23	18–25	20–25	17–21	20–26	14–16	18–22
Spines on rostellum	+	+	+	+	+	+	+
Spines on suckers	+	+	?	—	—	+	(+)
Position of genital pore	anterior	anterior	anterior	anterior	anterior	anterior	anterior
Cirrus pouch μ	114–115/50–73	105–114/41–46	89–121/40–65	105–121/48–54	130–180/54–85	57 (long ?)/90	120–140/50 (91–114/46)
Testes	28–30 7–9 poral	21–37 7–13 poral	33–35 11–15 poral	35–40	36–50 9–15 poral	22 6–7 poral	14–16 (21–23) (6–8 poral)
Egg capsules per proglottid	100–110	110–150	100–120	?	180–400	230	230–250 (200–220)
Eggs per capsule	1–4	1–4	3–4	?	1–4	2–5	1–4
Host	*Rattus assimilis*	*Rattus norvegicus*	*Rattus norvegicus* *Bandicota bengalensis*	*Rattus norvegicus*	*Rattus norvegicus*	Rat	*Rattus rattus*
Locality	Sth. Queensland	Hanoi	Rangoon	Rangoon	Manila	Canton	Tamatave

* All measurements in brackets have been found on re-examination of the original types

This is the first time that *R.* (*R.*) *celebensis* (Janicki) has been reported from Australia both from a child, and an autochtonous rat.* But since this tapeworm was first reported from *Lenomys meyeri* (Jentinck) from the Celebes, it very likely occurs throughout Australasia. *R.* (*R.*) *celebensis* has been found in rodents in the Philippines, Southern China, Formosa, Burma and Madagascar. It has been reported from man, in Siam (Leuckart, 1891), Formosa (Akashi, 1916), Philippines (Garrison, 1911, Africa & Garcia, 1934) and Queensland (present paper). It will, doubtless, be found elsewhere within this region, if searched for systematically.

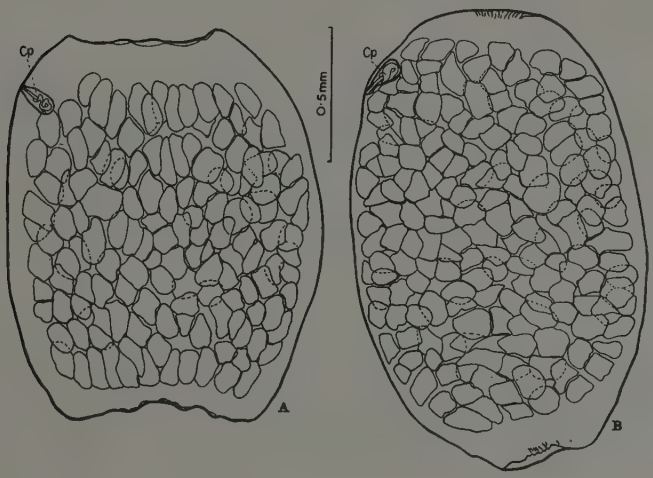
The species of *Raillietina* (*seu Davainea*) reported from man were first reviewed by Joyeux & Baer (1929) after an examination of the original material deposited in the Blanchard collection in Paris. They came to the following conclusions :

1. *Taenia demerariensis* Daniels, 1895, from British Guiana, is distinct from *T. madagascariensis* Davaine, 1869. An opinion that was later vindicated by the same authors (1940 ; 1949) (*vide infra*).
2. Under the name *T. madagascariensis* Davaine, 1869, there appear to have been described two distinct species : one from the Comores and Mauritius, and the other from Nossi Bé.
3. *T. madagascariensis* Garrison, 1911, from man in Manila, is a synonym of *R.* (*R.*) *celebensis* (Janicki) from rodents.
4. *T. madagascariensis* Leuckart, 1891, from a child in Bangkok, appears to be distinct from the above species. Its possible relationship to *T. madagascariensis* from the Comores, is discussed.
5. Several tapeworms belonging to the genus *Raillietina*, also occur in rodents, they may have been passed, secondarily, to man.

Subsequently, Tubangui (1931), reported a tapeworm from Philippine rats which is identical to the species described by Garrison (*loc. cit.*). Joyeux & Baer (1936) recorded a species of *Raillietina* found in *Rattus rattus* at Tamatave (Madagascar), which they were unable to identify with any of the species described from either the Comores or Mauritius.

* Ellermann (*Proc. Zool. Soc. Lond.* 1947, p. 262) is of the opinion that *Rattus assimilis* Gould is probably a race of *R. rattus*.

Re-examination of the fragments of the worms described by Joyeux & Baer (1929), from the collections of Blanchard and Davaine, has shown that in these the genital pore is always in the middle of the lateral border of the segment. This is in contradistinction to the position of the sexual aperture in *R.* (*R.*) *celebensis* which is invariably either in the anterior third of the lateral border in each



Gravid proglottides of *R.* (*R.*) *celebensis*: A.—from a Queensland Rat; B.—from a Queensland child. Cp.—cirrus pouch.

proglottid, or, as is more frequent, in the anterior quarter. Moreover, the fragments of the various strobilas reveal an internal anatomy not unique for the genus *Raillietina* but also identical with that of the genus *Inermicapsifer*. *Inermicapsifer* can be distinguished only in that all the species possess an unarmed scolex. They are found in rodents, hyraxes and man, in Africa*.

* *I. arvicanthidis* (Kofend), a frequent parasite of African rodents, has been reported at least three times from children in East Africa, and appears to occur quite commonly in man in Cuba (Baer, Kouri and Sotolongo, 1949; Baer, 1955). The first case observed by Davaine (1869) at Mayotte, was from a Creole child who had come from the West Indies!

Since Joyeux & Baer (1929) consider that the scolex, described by Blanchard as belonging to *T. madagascariensis*, is that of *T. saginata*, therefore no entire worm complete with scolex, has ever been described from the Comores or from Mauritius. In view of the above observations, we propose to consider the tapeworm from man, described in all textbooks under the name *Raillietina* (*R.*) *madagascariensis* as a *species sub judice* since it is at present, quite impossible to establish its true identity. In the hope of rediscovery of this tapeworm it would be necessary to obtain further material from either man or rodents, or both, from the Comores or Mauritius.

In the New World, Joyeux & Baer (1951) have established the existence of two species of *Raillietina* (*R.*) viz. *R.* (*R.*) *demerariensis* (Daniels, 1895) from man and a Howler monkey in the Guianas and Ecuador, and *R.* (*R.*) *alouattae* Baylis, 1947 from Howler monkeys in Surinam. At the time, the above authors overlooked a paper by Perez-Vigueras (1943) in which is described *R.* (*R.*) *halli* Perez-Vigueras, 1943 from a wild rodent in Cuba.*

Cameron & Reesal (1951) have described tapeworms from wild rodents from Trinidad, which they consider to be a variety of *R.* (*R.*) *demerariensis* which is named var. *trinitatae*. In a later paper, Stunkard (1953) reports this variety from a Venezuelean rodent and considers it to be identical with *R.* (*R.*) *demerariensis*, as redescribed by Joyeux & Baer (1951).

We have been able to examine numerous specimens of *R.* (*R.*) *halli* collected from the type host, *Capromys pilorides* Say in Cuba and have found this species to be identical with *R.* (*R.*) *demerariensis* (Daniels). On the other hand, the variety described by Cameron & Reesal is certainly not a synonym of *R.* (*R.*) *demerariensis*, as was suggested by Stunkard. Both the size of the rostellar hooks and the number of testes provide sufficient distinctive characters for separating them into two species. We therefore propose to raise the variety to specific rank and to name it *R.* (*R.*) *trinitatae* (Cameron & Reesal, 1951) *nov.comb.*

* It is interesting to find that in Cuba, wild rodents harbour tapeworms of the genus *Raillietina*, whereas man harbours a species of *Inermicapsifer* (*vide supra*) which has not yet been observed in any wild Cuban rodent.

The three species of *Raillietina* from mammals in the Neotropical region may be differentiated upon the following basis :

1. More than 80 testes per segment .....*R. alouattae* Baylis, 1947
   Less than 80 testes per segment 2

2. Rostellar hooks 10–18μ ; 26–46 testes
   R. trinitatae (Cameron & Reesal, 1951)
   Rostellar hooks 18–20μ ; 50–70 testes
   R. demerariensis (Daniels, 1895)

In the following the synonyms of these three species, and also those for *R.* (*R.*) *celebensis*, are listed, the host list and distribution given and the diagnosis of each species. The latter has been based, wherever possible, on examination of fresh material.

Raillietina (*R.*) *celebensis* (Janicki, 1902), Fuhrmann, 1920

Syn. *Davainea madagascariensis* Leuckart, 1891 *nec* Davaine, 1869 ; *Davainea celebensis* Janicki, 1902 ; *Davainea madagascariensis* Garrison, 1911 *nec* Davaine, 1869 ; *Davainea formosana* Akashi, 1916 ; *Raillietina* (*R.*) *celebensis* (Janicki), Fuhrmann, 1920 ; *Raillietina* (*R.*) *celebensis* var. *paucicapsulata* Meggitt & Subramanian, 1927 ; *Raillietina* (*R.*) *formosana* (Akashi), Joyeux & Baer, 1929 ; *Raillietina* (*R.*) *garrisoni* Tubangui, 1931 ; *Raillietina* (*R.*) *sinensis* Hsu, 1935 ; *Raillietina* (*R.*) *murium* Joyeux & Baer, 1936 : *Meggittia celebensis* (Janicki), Lopez-Neyra, 1943.

Hosts : Man, *Rattus rattus* L., *R. norvegicus* Berkenhout, *R. assimilis* Gould, *Bandicota bengalensis* (Gray & Hardw.), *Lenomys meyeri* (Jentink).

Distribution : Canton, Formosa, Hanoi, Rangoon, Tamatave, Manila, South Queensland.

Diagnosis : The length is 16–600 mm. with a maximum width of 2.5 mm. The scolex is 0.30–0.80 mm. in diameter. The rostellum which has a covering of small spines, bears 80–160 hooks, 14–26μ long. The four suckers are armed with minute spines and are 91–183μ in diameter. The genital pore is within the anterior third of the lateral border in each proglottid. The cirrus pouch is 89–180μ long and 40–85μ in diameter. There are 21–50 testes, 37–50μ in diameter, with the greater number on the aporal side of each

proglottid. The egg capsules are numerous (100–230) ; each capsule contains 1–4 eggs.

Raillietina (*R.*) *demerariensis* (Daniels, 1895), Joyeux & Baer, 1929.

Syn. *Taenia demerariensis* Daniels, 1895 ; *Davainea madagascariensis auctorum nec* Davaine, 1869 ; *Raillietina* (*R.*) *demerariensis* (Daniels), Joyeux & Baer, 1929 ; *Raillietina* (*R.*) *quitensis* Leon, 1935 ; *Raillietina* (*R.*) *brumpti* Dollfus, 1939 ; *Raillietina* (*R.*) *equatoriensis* Dollfus, 1939 ; *Raillietina* (*R.*) *leoni* Dollfus, 1939 ; *Raillietina* (*R.*) *luisaleoni* Dollfus, 1939 ; *Raillietina* (*R.*) *halli* Perez-Vigueras, 1943.

Hosts : Man, *Alouatta seneculus* (L.), *Capromys pilorides* Say.

Distribution : Ecuador, British Guiana, Cuba.

Diagnosis : The length is 90–120 mm. with a maximum width of 1.3–1.8 mm. The scolex has a diameter of 450μ. The rostellum is armed with 200–300 rostellar hooks, each 18–20μ long. The four suckers are armed with small spines. There are 50–70 testes in each segment. The cirrus pouch is 180–220μ long and 80–90μ in diameter. The egg capsules in the gravid segments are numerous (180). Each capsule contains 8–10 eggs.

Raillietina (*R.*) *alouattae* Baylis, 1947

Syn. *Raillietina* (*R.*) *multitesticulata* Perkins, 1950.

Hosts : *Alouatta seneculus* (L.), *A. macconnelli* Elliot.

Distribution : British and Dutch Guianas.

Diagnosis : The length is 130–340 mm. with a maximum width of 3.2–7 mm. The diameter of the scolex is 450–700μ. The rostellum bears 175–245 hooks, each 15–18μ long. The four suckers bear small spines. There are 110–140 testes per segment. The cirrus pouch is 220–308μ long and 100μ in diameter. The egg capsules are relatively few in the gravid segments (70–85) and each capsule contains 6–11 eggs.

Raillietina (*R.*) *trinitatae* (Cameron & Reesal, 1951) *nov.comb.*

Syn. *Raillietina* (*R.*) *demerariensis* var. *trinitatae* Cameron & Reesal, 1951 ; *Raillietina* (*R.*) *demerariensis* Stunkard, 1953 nec. Daniels, 1895.

Hosts : *Cuniculus paca* (L.), *Dasyprocta aguti* (L.), *Proechimys cayennensis* (Desm.).

Distribution : Trinidad, Venezuela.

Diagnosis : The length is 60–100 mm. and the maximum width, 1.3–2.7 mm. The diameter of the scolex is 270–370μ. The rostellum has 170–175 hooks, 10–11μ long. The four suckers are armed with minute spines. There are 26–46 testes in each segment. The cirrus pouch is 120–300μ long and 50–70μ in diameter. The egg capsules are numerous in the gravid proglottides (80–240). Each egg capsule contains 2–12 eggs.

Summary

Gravid proglottides of *Raillietina* (*R.*) *celebensis* (Janicki) are reported from a child in Brisbane, Queensland. Complete tapeworms of the same species are reported from *Rattus assimilis* Gould, from Mt. Glorious, South Queensland. A critical survey is made of all the cases previously recorded from man. *Raillietina* (*R.*) *madagascariensis* is for the present, regarded as a *species sub judice*. *Raillietina* (*R.*) *demerariensis* var. *trinitatae* Cameron & Reesal, 1951 has been raised to specific rank and named *R.* (*R.*) *trinitatae* (Cameron & Reesal, 1951) *nov.comb.* The synonyms of *R.* (*R.*) *celebensis* ; *R.* (*R.*) *demarariensis* ; *R.* (*R.*) *alouattae* and *R.* (*R.*) *trinitatae*, are discussed.

Acknowledgment

We are indebted to Dr. M. J. Mackerras for forwarding the Queensland material examined and identified.

References

Africa, C. M. and Garcia, E. Y., 1934.—"A Rat Tapeworm (*Raillietina garrisoni* Tubangui, 1931) transmissible to Man. With notes on *Davainea madagascariensis* Garrison, 1911." *Philipp. J. Pub. Health*, **1**, 44–51.

Baer, Jean G., 1955.—"Un nouveau cas de parasitisme d'un enfant en Afrique orientale par le cestode *Inermicapsifer arvicanthidis* (Kofend, 1917)." *Acta trop., Basel*, **12**, 174–176. (W.L. 121a)

BAYLIS, H. A., 1947.—"Some Roundworms and Flatworms from the West Indies and Surinam, II. Cestodes." *J. Linn. Soc.* (*Zool.*), **41,** 406–414. (W.L. 11296)

CAMERON, T. W. and REESAL, M. R., 1951.—"Studies on the endoparasitic fauna of Trinidad Mammals, VII. Parasites of Hystrichomorph Rodents." *Canad. J. Zool.*, **29,** 276–289, 24 figs.

*JOYEUX, Ch. and BAER, J. G., 1929.—"Les cestodes rares de l'homme." *Bull. Soc. Pat. exot.*, **22,** 114–136. (W.L. 5310)

———— 1936.—"Sur quelques Cestodes de Madagascar. *ibid*, **32,** 39–43, 2 figs.

———— 1940.—"Anatomia y posicion sistematica de *Raillietina* (*Raillietina*) *quitensis* Leon, 1935. Cestode parasito del Hombre." *Rev. Med. trop., Havana*, **6,** 79–88, 3 figs. (W.L. 18902a)

———— 1949.—"L'hôte normal de *Raillietina* (*R.*) *demerariensis* (Daniels, 1895) en Guyane hollandaise." *Acta trop., Basel*, **6,** 141–144. (W.L. 121a)

LOPEZ-NEYRA, C. R., 1943.—"Las Raillietinas parasitas humas." *Rev. ibér. Parasit.*, **3,** 141–168 ; 257–258, 3 pls. (W.L. 18858c)

PEREZ-VIGUERAS, I., 1943.—"Un genero y cinco especies nuevas de helmintos cubanos." *Univ. Habana*, 41 pp. 19 figs.

PERKINS, K. W., 1950.—"A new Cestode, *Raillietina* (*R.*) *multitesticulata* n.sp. from the Red Howler Monkey." *J. Parasit.*, **36,** 293–296, 6 figs. (W.L. 11428)

STUNKARD, H. W., 1953.—*Raillietina demerariensis* (Cestoda), from *Proechimys cayennensis trinitatus* of Venezuela. *ibid.*, **39,** 1–8, 4 figs.

TUBANGUI, M., 1931.—"Worm parasites of the Brown Rat (*Mus norvegicus*) in the Philippine islands, with special reference to those forms that may be transmitted to human beings." *Philipp. J. Sci.*, **46,** 537–591, 19 figs. (W.L. 16189)

* This paper contains all the literature cited prior to 1929.

Journal of Helminthology, *Vol.* XXX, *No.* 4, 1956, *pp.* 183-188.

Mirandula parva gen. et sp. nov. (Cestoda, Dilepididae) from the Long-nosed Bandicoot (*Perameles nasuta* Geoff.)

By Dorothea F. Sandars*

Amongst helminths collected and forwarded by Dr. M. J. Mackerras of the Queensland Institute of Medical Research, Brisbane, were fifteen very small cestodes (some not entire) from the duodenum of a long-nosed bandicoot, *Perameles nasuta* Geoff., from Mt. Glorious, South Queensland. They were attached to the mucosa of the duodenum.

Material and Methods

The specimens were fixed in Bles (formol-acetic-alcohol) and preserved in 70% alcohol. They were stained in hydrochloric-alcoholic-carmine and mounted in canada balsam ; two specimens were stained and then mounted in berlase medium. All measurements were made from these mounted specimens. It was impossible to cut sections of the present material.

Description

Each tapeworm consists of a scolex, a very short "neck" region and only four segments : one immature, one mature, one with developing uteri and one gravid (Fig. 1). The length of the whole worm including the rostellum, which in most specimens is partly or wholly protruded, is 1.00–1.41 mm. The rostellum, together with its sheath, is 0.68–1.12 mm. long and usually between two-thirds and four-fifths of the total length of the worm. The length of the worm from the apex of the scolex to the posterior extremity of the gravid proglottid is 0.69–0.89 mm. The greatest body width is frequently across the scolex, which has a maximum diameter of 0.32–0.43 mm. across the suckers. In a few specimens the maximum diameter of the scolex is the same width as the maximum width of the rest of the body. There are some variations e.g. in one worm the maximum

* Institut de Zoologie, Université de Neuchâtel and Department of Parasitology, London School of Hygiene and Tropical Medicine, while on study leave from the University of Queensland and the Queensland Institute of Medical Research, Brisbane, Australia.

body width, i.e. 0.41 mm., is across the segment with the developing uteri ; in another specimen the maximum body width of 0.4 mm. is across the gravid proglottid. In the latter, the diameter of the scolex and the maximum width of the mature segment is 0.39 mm. Following are the measurements of the four segments of the strobilae, in each the maximum width and length being given : immature segment, 0.30–0.39 mm. × 0.05–0.07 mm., mature segment, 0.32–0.42 mm. × 0.07–0.11 mm., penultimate segment, 0.32–0.42 mm. × 0.07–0.09 mm., gravid segment 0.33–0.40 × 0.16–0.27 mm. The segments increase considerably in relative length along the strobila, this being especially evident in the gravid proglottides.

As has already been noted, the scolex is a very prominent part of this cestode. It has a maximum length of 270–320 μ. The four suckers are very weakly developed and there appears to be no trace of spines on them. They measure 160–183 μ × 125–168 μ and have a depth of 55–78 μ. The rostellum is an extremely large structure and it is obvious that it is the most important organ for attachment of this parasite to the mucosa of the duodenum of the host. The rostellar sac is very thick-walled and extends posteriorly into the middle of either the mature segment or even into the penultimate segment of the strobila. This sac is 388–571 μ long and varies in diameter, increasing from 46–50 μ posteriorly to 91–114 μ anteriorly. In all specimens there is a marked enlargement of the rostellar sac, as it passes towards the region where the rostellum emerges at the anterior end of the scolex (Fig. 1). The rostella are seen in several different positions of protrusion among the specimens examined (Figs. 1, 2, 3). The maximum diameter of the protruded rostellum is 68–105 μ. At its distal end it expands to form a knob-like structure which is 87–113 μ wide and 45–69 μ long. This bears a double row of 36 hooks, one row of hooks being 37 μ long and the other row 41–43 μ (Fig. 4).

Since no sections could be cut it has not been possible to make any detailed observations on the muscular system of the body. It is however apparent from the whole specimens that the musculature is only very weakly developed in all the proglottides.

On both sides of the strobila, the dorsal and ventral longitudinal excretory canals are very narrow ducts joined posteriorly in each proglottid by transverse canals.

In every segment there are two sets of reproductory organs which

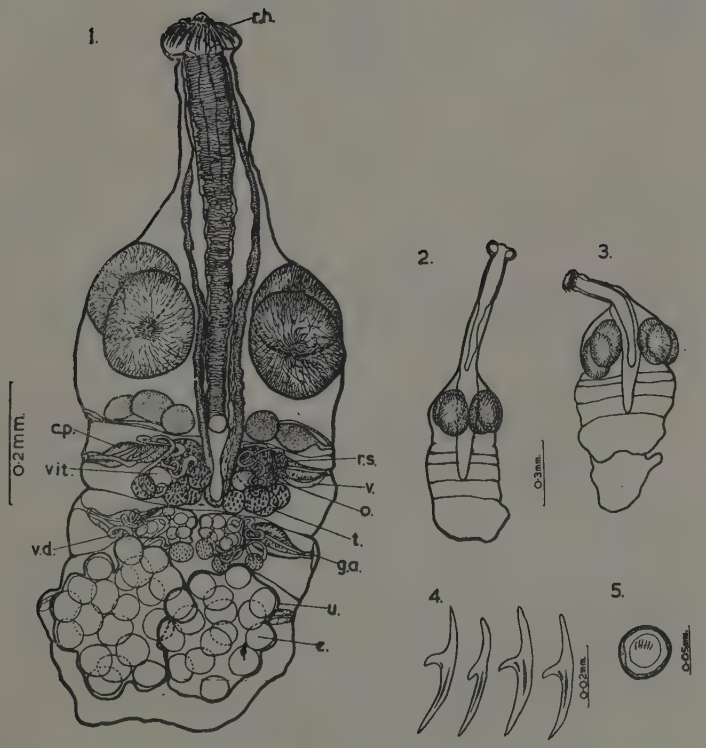


Fig. 1. *Mirandula parva* n. gen., n.sp. Whole mount specimen showing details of anatomy. Fig. 2. Whole mount specimen showing rostellum fully extended. Fig. 3. Whole mount specimen showing gravid segment sloughing off. Fig. 4. Rostellar hooks.

ABBREVIATIONS

e.p.—cirrus pouch; **g.a.**—genital atrium; **o.**—ovary; **r.h.**—rostellar hooks; **r.s.**—receptaculum seminis; **t.**—testes; **v.**—vagina; **vit.**—vitellarium.

in the immature and mature segments, and sometimes also in the segment with the developing uteri, are separated by the median rostellar sac (Fig. 1). In each male reproductory system there are four spherical testes, each 36–43 μ in diameter. The vas deferens is a very long sinuous duct which is comparatively wide, so that its entire length may serve for the storage of sperms. It makes a number of close coils before entering the cirrus pouch, within which it lies in several loose coils. The proximal end of the cirrus pouch reaches the ventral longitudinal excretory canal; it is 52–91 μ long × 30–32 μ in diameter. The cirrus is armed with small spines and is approximately 30–45 μ long. No external or internal vesicula seminalis is present. The cirrus pouch opens into a long narrow genital atrium. The genital pore is either mid-lateral in the anterior half of both sides of each proglottid or in the anterior half of each side of the proglottid. Sometimes it may be in the one position on one side of the proglottid and in the other position on the other side.

In each female reproductory system the vagina opens into the genital atrium close to the cirrus opening. The relative position of the vagina and the vaginal pore may be different in the two sets of reproductory organs of the one proglottid. Usually in one, the vagina lies posteriorly to the cirrus pouch while in the other, the vagina and its pore are in a more anterior position. The vagina enlarges to form a receptaculum seminis which measures 20–30 μ × 27–35 μ. It usually lies close to the proximal end of the cirrus pouch, towards the midline of the strobila. The ovary is usually spherical measuring 45–69 μ in diameter or it may be sub-spherical, measuring from 45–57 μ × 48–64 μ. The vitellarium is spherical, 30–34 μ in diameter. No trace of either uterus is seen in the mature segment, but in the penultimate segment of the strobila, the two uteri are already developed as transversely elongate sacs measuring 59–73 μ × 91–101 μ, within which are small immature eggs 23–27 μ in diameter.

In the gravid proglottid the two uteri are filled with eggs. Each uterus contains between 16–24 eggs. There are usually 32–42 eggs altogether in each gravid proglottid. The two uteri of the gravid proglottid become so distended that frequently it is difficult to differentiate them (Fig. 1). In one specimen the gravid proglottid is in the process of separating from the strobila and already the proglottid immediately behind it is almost completely gravid (Fig. 3). The two cirrus pouches are still present in each gravid proglottid, in which also testes may sometimes still be seen lying posteriorly.

The eggs within the uteri of the gravid proglottid are sometimes not mature and then measure 30–35 μ in diameter. The mature eggs measure 41–52 μ in diameter, have a fairly thick shell and contain an onchosphere 32–45 μ in diameter, within which six well defined hooks 11–14 μ in length are present (Fig. 5.).

Discussion

The presence of an armed rostellum, unarmed suckers, and a sac-shaped uterus places the genus *Mirandula* within the family Dilepididae Fuhrmann, 1907, and in the sub-family Dilepidinae Fuhrmann, 1907.

It may be of value to note here that *Mirandula* is precluded from belonging to the family Anoplocephalidae Fuhrmann, 1907, because of the presence of the rostellum, the shape of the segments, and the persistent sac-like uterus ; the shape of the rostellar hooks prevents its inclusion within the family Davaineidae Fuhrmann, 1907 ; since both internal and external vesiculae seminales are absent and the vas deferens is a long duct, it therefore cannot belong to the family Hymenolepididae Fuhrmann, 1907.

It is believed to be the first record of a double-pored tapeworm within the sub-family Dilepidinae Fuhrmann, 1907. It is interesting that it has been recovered from one of the bandicoots from Australia belonging to the Perameloidea which is perhaps one of the more primitive groups of the Marsupialia. All bandicoots are omnivorous feeders, their diet including a number of insects (Sandars, 1952). T. H. Johnston, 1911, described a member of the Dilepidinae, *Bancroftiella tenuis* from the intestine of a black wallaby, *Macropus ualabatus* from Victoria, Australia. Several other species of this latter genus have subsequently been described from Australia.

Mirandula n.gen.

Generic Diagnosis : Very small, double-pored cestodes, each strobila having only a few proglottides. There are four spherical testes in each set of reproductory organs. The scolex has four weakly developed suckers and an extremely large retractile rostellum bearing a double crown of hooks. The genital ducts pass dorsally to both longitudinal excretory canals. Each uterus is developed as a transverse sac and persists as a slightly lobed sac. Eggs relatively large, with a fairly thick shell. Adults, parasites of Marsupials.

Distribution : Eastern Australia.

Mirandula parva n.sp.

Specific Diagnosis : The strobila is 1.0–1.4 mm. long and has four proglottides. The length of the rostellum and its sac is usually greater than two thirds of the entire body length. There are 36 rostellar hooks, 37–40 μ long, arranged as a double crown. The testes are 36–43 μ in diameter, lying transversely in the proglottid and posteriorly to the female reproductory organs. The vas deferens is a wide conspicuous duct winding throughout one half of each segment. The ovary is typically spherical. A receptaculum seminis is present. The mature eggs are 41–52 μ in diameter and the onchospheres are 41–52 μ ; six hooks are evident in these eggs.

Type and paratype specimens are lodged in the Queensland Museum, Brisbane, Australia.

Summary

Mirandula parva n.gen., n.sp., is described from the long-nosed bandicoot, *Perameles nasuta* Geoff., from Mt. Glorious, South Queensland. This is the first double-pored genus within the sub-family Dilepidinae Fuhrmann, 1907, of the family Dilepididae Fuhrmann, 1907.

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References

Johnston, T. H., 1911.—"The Entozoa of Monotremata and Australian Marsupialia. No. 2". *Proc. Linn. Soc. N.S.W.*, **36,** 47–57. (W.L. 16790a).

Sandars, D. F., 1952.—"Bandicoot Food". *Proc. roy. Soc. Qd.*, **62,** 33. (W.L. 16903).

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*Observations on the Life Cycle of *Davainea proglottina* in Britain

By A. H. Abdou, B.V.Sc., Ph.D.

From the Department of Parasitology,
London School of Hygiene and Tropical Medicine

In *Davainea proglottina* as in most of the other cestodes the life cycle includes an intermediate host. That the intermediary host might be a slug was originally suggested by Grassi and Rovelli in Europe (1888). Although they admitted that they had been unable to control their work with due care, they identified the following slugs as intermediate hosts for this cestode : *Agriolimax agrestis*, *Limax cinereus* and *Limax flavus*. They found experimentally that the adult stage was reached in eight days in chickens after the ingestion of infected slugs.

In nature, the host harbouring the infection passes the gravid segments with its droppings. These are eventually eaten by the correct intermediate host inside which the cysticercoids develop. On the slug being swallowed by a fowl, the cysticercoid develops into the mature worm.

Grassi and Rovelli (1888) also suggested that direct infection of poultry with *D. proglottina* might take place. A. Railliet and A. Lucet (1892), trying to verify this suggestion fed two healthy birds with gravid segments of this worm mixed with wet bread. For some reason the birds died, the first after 25 days and the second after 31 days ; no *D. proglottina* were recovered from either. Railliet and Lucet explained the presence of heavy infection with *D. proglottina* in poultry where very few limacinae exist by the possibility that other molluscs may act as intermediate hosts.

In his experiment to work out the life cycle of this parasite, Meggitt (1916), at Birmingham, got only negative results. He said that, although the slugs undoubtedly ate the tapeworm, dissections and sections never showed the slightest trace of developing cysticercoids. Altogether 5 *Arion ater* L., 6 *Arion hortensis* Fer., 18 *Arion circumscriptus* John, and 45 *Agriolimax agrestis* L. were fed and examined, the time elapsing between the first date of feeding and the dissection varying from 10–35 days.

* Part of a Thesis approved by the University of London for the award of the Ph.D. degree.

Joyeux (1920), failed to infect experimentally seven *Agriolimax agrestis* with *D. proglottina*. These were twice fed on gravid segments spread on a piece of lettuce.

Chandler (1923), in the U.S.A. found 150 cysticercoids in one naturally infected *Agriolimax agrestis* collected from a chicken yard. The cysticercoids were fed to two chickens and after twenty days their droppings contained the gravid proglottides of *D. proglottina*. He also successfully infected two *Agriolimax agrestis* in which the mature cysticercoids were found after twenty-two days. On trying to infect four *Limax flavus*, only one cysticercoid was found in one of them ; the others were negative.

Kalkus (1928), was successful in infecting four birds with *D. proglottina* from cysticercoids obtained from *Limax cinereus*.

Bisset (1928) in England again failed to infect *Agriolimax agrestis*.

Sawyer and Hamilton (1929) reported *Prophysaon andersoni* as an intermediate host for the tapeworm.

Myrna Jones (1929), U.S.A., experimentally infected the following molluscs : *Zonitoides arborea, Vallonia indentata, Gastrodonta ligera* and *Polygyra thyroides*, and then fed them to clean chickens from which the mature worms were recovered. Later in 1930 she again succeeded in infecting the snail, *Physa heterostropha*.

Wetzel (1932), in Germany, under accurate experimental conditions, not only confirmed the transmission of *D. proglottina* by means of the slugs (*Limacidae*) previously reported by Grassi and Rovelli, but also by the *Arionidae, Arion empericorum, Arion hortensis, Arion circumscriptus* and *Arion intermedius*, and among the *Helicidae, Cepea nemoralis*.

Bayon (1933), collected slugs (*Agriolimax agrestis*) in a field where no chickens were present, and fed them on washed proglottides of *D. proglottina*. He stated that there were positive results, but there were also numerous failures extending over months of work, due to the difficulty of keeping slugs alive under laboratory conditions.

Taylor (1933), reported that naturally infected snails and slugs were collected at the South-Eastern Agricultural College and fed to chickens in varying doses between 29 and 304. Adult *D. proglottina* were recovered from the intestine (3,000–4,000 in each bird). These snails were *Trichia sericea, Helicella virgata, Helicella caprata* and the slugs, *Agriolimax agrestis*.

Levine (1938), experimentally infected chickens by feeding them on slugs (*Agriolimax agrestis*) harbouring the mature cysticercoids.

The number of worms recovered from three birds was 5,617; 2,595 and 1,932 respectively.

Material and Methods

Intermediate Hosts

Slugs : These molluscs are soft-bodied and vary considerably in size when full grown. The greater part of the body comprises the foot, an elongated muscular structure with a flat under surface, which functions as an organ of locomotion. Slugs are hermaphrodite, although self-fertilisation does not generally take place.

Differentiation of the Slugs

Correct specific identification is of extreme importance. In addition to certain points concerning the species and their recognition, two keys, Barnes and Weil (1943) and Quick (1949), were used for the separation of the common genera, and several references were made to Taylor's Monograph and to other living authorities.

For distinguishing the different species, the writer depended on the general description of the individuals, and to some extent on their colour and size. In doubtful cases the internal anatomy was examined.

After some time, due to the frequency of handling these slugs, it was found that certain external features were of great help in distinguishing the different species by the naked eye. The aid of a hand lens was rarely needed. The following observations were made on the different species found.

Arion circumscriptus. When contracted, the transverse section is bell-shaped or otherwise depressed or flattened ; the right mantle band arches high over the respiratory orifice ; opaque white sole.

Arion hortensis. Orange or yellow sole.

Arion ater. When contracted, it has a hemispherical shape, and if touched, will roll and sway its body from side to side.

Milax gracilis. The sole is tricoloured, and characterised by the series of chevron-like grooves with apices directed backward when in motion. It is noticeably sticky.

Milax sowerbyi. The keel is lighter than the back (often orange in colour) and abruptly angulated.

Agriolimax reticulatus. Short truncated keel. Milky mucus.

Limax flavus. Blue tentacles, yellow mottled mantle.

Limax maximus. Large size. Mantle not banded, marbled or spotted with black. Short keel, not prominent. Small tubercles.

Limax cinereo-niger. Its coarse tubercles and long keel are more prominent. No spots or bands on the mantle.

Studies of the Slugs Under Natural Conditions

It was found that it is of extreme importance to study the behaviour of the intermediate hosts in nature with regard to their normal habitat, food requirements, and ecology in order to gain information about the keeping of the slugs under laboratory conditions.

This work was carried out at Winches Farm Field Station of the London School of Hygiene and Tropical Medicine at St. Albans. Slugs were collected from their natural habitat—moist, shady places, under stones, logs and wood, and on the leaves of plants, e.g. cabbage and lettuce. Slugs were often found buried in the ground, e.g. *Arion circumscriptus*. Large pieces of wood, sacks, kept moist with water in the dry season, were put at different places ; this proved to be an easy and successful way to concentrate slugs for collection. With experience, it is possible to know where and when to look for and find these slugs. Several hundreds can be collected in one hour after a good shower of rain, while only a few can be found in dry weather. The writer concluded from this that the weather has a profound effect on the abundance and activity of these slugs.

According to R. Carrick (1947), the soils of high water-holding capacity sustain the densest population of slugs. Although the density of the slugs varied seasonally, I observed that the slugs most abundant and most prevalent at Winches Farm were *Agriolimax reticulatus* and *Arion hortensis* ; these seem to be present all over the country. Their presence has been reported by Miles, Wood and Thomas (1931) in Manchester and Lancashire ; by Barnes (1943) in Harpenden ; and by Carrick (1948) in Scotland. Others, such as *Milax gracilis*, *Arion circumscriptus* and *Arion ater*, were less frequent. It is of interest to note here that the colours, shades and sizes of slugs of the same species differed greatly. A record was kept of the plants on or near which these slugs were found. These were cabbage, lettuce, potato, stinging nettles and green grass.

Feeding Habits

Slugs (e.g. *Arion hortensis* and *Milax gracilis*) usually feed at

or near the surface of the soil, eating the lower or fallen leaves of cabbages, leeks and lettuces. Also, quite a number of slugs, especially the large ones, e.g. *Arion ater*, live on green grass.

Generally speaking, slugs eat most of the winter green plants, decaying vegetable matter, fungi, potato, algae, etc. At Winches Farm there was a field of cabbage, where it was noticed that hundreds of slugs, especially *Arion hortensis* and *Milax gracilis*, lived on the tubers and leaves of plants.

In the laboratory the slugs were fed on clean lettuce washed in tap water for 10 minutes, slices of potato, carrots, cooked and uncooked, and bread. The slugs were attracted by the faeces of poultry, on which they seemed to be feeding. It was also noticed how easily these slugs, especially *Agriolimax reticulatus*, devoured the proglottides, while on the other hand, snails did not show any of these habits. This finding agrees with Lebour (1915), who states that slugs eat tapeworms on the field, and are exceedingly fond of proglottides of *Monezia* and *Cittotaenia*, and also rabbits' faeces.

The slug's activity appears to depend mainly on the degree of moisture in the soil. In the laboratory the slugs did not keep well when the soil was too damp, and so the turf in the large glass vessels where they were kept was sprayed with only a small amount of water daily. The distribution and number of different slugs depended to a large extent on the surroundings and availability of food material. Near a woodland (Oaklands near Winches Farm) *Arion subfuscus* and *Limax maximus*, which feed mainly on fungi were found. Near cabbage and turnips, one finds *Arion hortensis* and *Milax gracilis*, while *Agriolimax reticulatus* was found nearly everywhere in the open field, particularly under damp leaves, stones and logs, as well as on lettuce. *Limax flavus* was found near cellars and drainpipes in the gardens. The replacing of the stones and logs from under which slugs were recovered facilitated further collecting. These objects seem to attract the slugs. It was noticed that some slugs escaped through surprisingly narrow slits which could barely be seen, and it was important to put some vaseline around the rim of the glass vessels and to adjust the tops firmly. Decayed lettuce and cabbage were removed daily from the containers, as they proved to be harmful to the slugs owing to the formation of hydrogen sulphide.

Collecting Slugs

To exclude natural infection with *D. proglottina* slugs were collected from the Farm's field, from gardens and from parks to

which poultry had no access. To ensure that these slugs were not harbouring *D. proglottina* cysticercoids, a percentage of the specimens were always dissected, while others were kept as control until the end of the experiment. Neither in the first group nor in the control group were any *D. proglottina* cysticercoids detected during all the experiments.

Later it was possible to keep and breed these slugs under laboratory conditions for use in further experimental work.

The slugs recovered from Winches Farm were :—

Arionidae : *Arion ater, Arion circumscriptus, Arion hortensis, Arion subfuscus, Arion intermedius.*

Milacidae : *Milax gracilis, Milax sowerbyi.*

Limacidae : *Limax maximus, Limax cinereo-niger, Agriolimax reticulatus.*

From a garden in the West of London, *Limax flavus* was collected. Some slugs, which proved to be *Agriolimax carvanae,* were received from Liverpool.

Methods of Keeping Slugs Alive

In order to use slugs for experimental purposes one has first to work out methods of keeping them alive. Meggitt (1916) tried to keep slugs in large earthenware pots. The bottoms of these pots were covered with moist earth upon which stones were placed. The escape of the slugs was prevented by covering the tops of the pots with fine muslin and the pots kept in total darkness. Cabbage leaves and potatoes were proved to be the best food. He admitted that in spite of all care culture after culture died off.

Bayon (1933) reported that he kept infected slugs on wet blotting paper in glass dishes and fed them on chopped cabbage. He stated that although mortality was heavy, a few survived long enough to develop mature cysticercoids which he fed to two chickens.

The writer tried out Bayon's method at first. Slugs which had been exposed to infection were divided into batches of ten each and were kept in large glass Petri-dishes (6 in. $\times \frac{1}{2}$ in.). The insides of these dishes were covered with damp filter paper which was changed daily. This method had the advantage of drawing attention to the bodies of newly-dead slugs, which were later found to be harbouring

cysticercoids in different stages of development. However, it was found that a great deal of time was taken up in changing the filter-papers of the Petri-dishes daily, when sometimes up to as many as 20 dishes at a time were used. This method was abandoned and after studying the natural habitats of the slugs they were kept as far as possible under conditions approaching those in nature. For this purpose the top of a wooden box (2 ft. × 2 ft. × 1 ft.) was removed and the box was filled with damp earth to a depth of 4 in. A wet sack was placed over the earth. The box was placed near a pond and under a tree in the field at Winches Farm, and was slightly raised from the ground by small stones placed beneath each corner. The earth under the box was thus kept grass-covered and always moist. These conditions were found to be ideal for attracting and keeping slugs for the experimental work. Food was supplied and in dry weather the earth in the box and the surrounding turf were sprayed with water.

In the method used inside the laboratory, the floors of three glass vessels measuring 3 ft. × 2 ft. × 2 ft. in height were covered with a piece of turf, on top of which stones were placed. These large vessels were kept dark by covering the sides and top with paper. Water was sprinkled daily to keep the turf just moist. It was found that, although moisture is of great important in the production of their slime, excessive dampness is harmful to the slugs. They were fed on clean lettuce, cabbage and sliced potatoes. These containers were utilized to house the slugs after they had been fed on the gravid proglottides of *D. proglottina*. A satisfactory number of these slugs (about 60%) usually survived long enough to allow the development of the cysticercoids, and some of them often started egg-laying. The tops of these vessels were tightly covered with large pieces of glass to prevent the slugs from escaping.

Breeding of the Slugs

The slugs which were collected from the Farm's field and kept in the laboratory, bred well in the large glass containers. Their eggs, which are translucent and round in shape, were often found under loose turf in clusters of varying numbers. When these eggs were left undisturbed and damp they hatched in about three or four weeks. The temperature was about 21°C. Barnes (1943), states that the eggs of the slugs hatch in three to six weeks. Carrick (1941), reported that the time of development of the eggs of the slugs varies from 105 days at 5°C to 18 days at 20°C.

When eggs were found while collecting slugs in the field they were carefully transferred to the containers. When the eggs hatched

the young slugs were removed when about a month old to a special container and were available for experiments when required. These newly-hatched slugs were extremely small in size and did not achieve normal size until they were about three months old.

Miles (1931), stated that conditions of temperature and food supply determine the length of time required to reach maturity, and that this varies from six to eight months.

The following slugs were successfully bred by the writer under laboratory conditions : *Agriolimax reticulatus*, *Arion ater*, *Arion hortensis* and *Arion circumscriptus*.

Methods of Infecting the Slugs

The slugs were fasted for 24 hours before the experiment. After collecting the freshly passed gravid proglottides they were squashed, cut in pieces with a very sharp scalpel, and shaken vigorously in a small amount of distilled water to provide an even distribution of eggs in the emulsion. Some drops containing the viable eggs were scattered on a very small piece of lettuce and placed inside a glass tube (1 in. $\times$ 2 in.) lined with moist filter paper. One slug was kept overnight in each tube which was closed with a cork. In earlier experiments a few proglottides were put on the piece of lettuce but a number of the slugs did not become infected although the lettuce was eaten. It was thought that this was due to the fact that the segments might have moved off the lettuce. The chance of infection was increased as a result of the spreading of the eggs on the pieces of lettuce.

Although some difficulty was encountered, the slugs were always induced to eat the lettuce. Slugs of different ages were fed, and it was found that the younger ones were more susceptible but *Agriolimax reticulatus* of almost all ages became infected. A number of slugs did not become infected. This may have been due to the fact that not all the slugs and snails ate the eggs of *D. proglottina*, in spite of their being spread on the food ; or because some of the eggs were immature.

The feeding of slugs with proglottides was carried out on a glass plate under a low power binocular microscope in order to observe how many proglottides each individual slug received. This procedure proved to be very time-consuming and often needed great patience. However, it had the advantage of providing accurate information as to the number of segments eaten and moreover, the exact time when the slug had eaten them.

The slugs were kept under natural conditions, as far as possible,

inside the laboratory, as previously mentioned. The temperature was about 21°C and the slugs were fed on washed clean lettuce, cabbage and potatoes. A number of the slugs died at different intervals. These were always dissected to determine the different stages reached by the cysticercoids and the shortest time needed to reach full maturity. Under these conditions, it took 18–19 days.

Results

In the determination of the suitability of the various slugs to act as intermediate hosts for *D. proglottina* the following species were used.

Family Limacidae

I. *Subfamily Limacinae*

Agriolimax reticulatus (Common field or Grey slug)

This slug is the most prevalent and widely distributed in Great Britain. It also proved to be a very suitable intermediate host for *D. proglottina* for as many as 500 cysticercoids were recovered from individually infected specimens. Slugs of nearly every age were experimentally infected with almost 100% results. This species is recorded for the first time as an intermediate host for the poultry tapeworm *D. proglottina*. *Agr. reticulatus* like all the other species of *Agriolimax* crawls swiftly and being the commonest and most ubiquitious slug makes it the most important intermediate host in this country.

Agriolimax carvanae

Although the writer had only 20 specimens of this slug sent from Lancashire, feeding experiments produced a very large number of cysticercoids in almost 100% of these slugs. This species has hitherto not been recorded as an intermediate host for *D. proglottina*.

Limax maximus (Great Slug)

Several fully grown specimens (12–14 cm.) were fed on freshly collected gravid proglottides but no cysticercoids were detected after three weeks. Four very young specimens (5–6 cm.) were fed with the same result.

Limax cinereo-niger (Ash-black slug)

This slug was reported by several authors to be an intermediate host for *D. proglottina*. Of several specimens fed on gravid segments,

in only one case, that of a very young specimen (5 cm.) was the infection successful. The rarity of this species and the low percentage of infection makes it of very small importance as an intermediate host in this country. Furthermore, since *L. cinereo-niger* may be considered the largest slug found in England (specimens up to 18 cm. were found), it cannot be eaten by fowls.

Limax flavus (Yellow slug)

Opinions on the importance of this species as an intermediate host for *D. proglottina* diverge ; some authors, Grassi and Rovelli describe it as a rare intermediate host ; Chandler (1923) reported it as a very slightly susceptible intermediate host, having obtained one cysticercoid after feeding a large number of oncospheres to four specimens. On the other hand, Wetzel (1932) succeeded in infecting 37 slugs from which numerous fully developed cysticercoids were obtained and considered the slug a very suitable intermediate host for this tapeworm. None of this species was found in the open field or in poultry runs. Only two specimens (7–8 cm.) were collected near a cellar and after feeding them the gravid segments, both showed few mature cysticercoids. Because of its unusual habitat, *L. flavus* should not be considered as of great importance in practice. This species has not been recorded previously in this country as a probable intermediate host.

II. *Subfamily Parmacellinae*

Milax gracilis ("Sticky back")

This is one of the so-called "Keeled slugs" which is quite common in the area. Eighty-two slugs of this species were fed on gravid segments and only the young ones (3 cm.) developed numerous cysticercoids. This slug is so noticeably sticky that the fowls do not seem to like it very much and this might lessen its importance as an intermediate host for *D. proglottina*. This species is recorded for the first time as an intermediate host.

Milax sowerbyi (Keeled slug)

This is a larger species than the previous one (8 cm.). Forty-six slugs were fed on gravid segments of *D. proglottina*. Three weeks later mature cysticercoids were recovered only from the young specimens (0.4 cm.). This species is recorded for the first time as an intermediate host of this tapeworm. *N.B.* Although feeding produced numerous cysticercoids in both *M. gracilis* and *M. sowerbyi*, the percentage of individual infection was about 60%.

Arion hortensis (Garden slug)

This species has not been recorded previously in this country as an intermediate host for *D. proglottina*. Meggit (1916) failed to find any cysticercoids after feeding a number of these slugs on gravid segments. Wetzel (1932) only infected one slug recovering numerous cysticercoids. Hundreds of this species (1–2 cm.) were fed freshly collected gravid proglottides and enormous numbers of cysticercoids were recovered. Almost 100% of these slugs of all ages were successfully infected.

A. hortensis being very common all over the British Isles and very susceptible to infection makes it another very important intermediate host for *D. proglottina* in Great Britain.

Arion ater (*A. empericorum*)

Meggit (1916) found no cysticercoids in this slug. Wetzel (1932) fed 28 slugs on gravid segments but recovered cysticercoids from only one. During these present experiments only the young slugs (3–5 cm.) proved to be susceptible. Twenty-four of these were fed on a large number of oncospheres and all the survivals showed mature cysticercoids within three weeks. A number of fully grown *A. ater* (10–14 cm.) were fed the gravid proglottides of *D. proglottina* but no cysticercoids were detected in them. This species has not been recorded previously in Great Britain as an intermediate host.

Arion subfuscus (Dusky slug)

This slug appears to be rare at Winches Farm. Feeding five mature specimens (8 cm.) and three young ones (4 cm.) showed no cysticercoids.

Arion circumscriptus (The Bourguignat's slug)

Meggit (1916) failed to recover any cysticercoids after feeding six slugs the gravid segments while Wetzel (1932) reported obtaining the cysticercoids from young slugs. After feeding several young specimens (2 cm.) only a few cysticercoids were recovered from certain individuals while several full grown slugs (4–5 cm.) failed to show any cysticercoids. As *A. circumscriptus* is common and widely distributed all over the British Isles it should be considered as a possible intermediate host for *D. proglottina*.

This species has not been recorded previously in this country as an intermediate host.

Arion intermedius (Hedgehog slug)

Only two specimens (1.5–2 cm.) were fed the gravid proglottides of *D. proglottina*. In one a number of mature cysticercoids were found while the other was negative.

This species has not been recorded previously in this country as an intermediate host.

Family Helicidae

Cepea nemoralis

Wetzel (1932) succeeded in infecting young specimens and stated that in fully grown snails development rarely occurred. The gravid segments of *D. proglottina* were fed to twenty-six *C. nemoralis* (5 cm. × 1.5 cm.) by the writer but in no case were any cysticercoids detected.

Cepea hortensis

With regard to this species seventeen snails (6.5 cm. × 2 cm.) were fed freshly collected gravid segments with negative results.

To ascertain whether the slugs would eat the chickens' droppings when there was an abundance of normal diet present, the following experiments were carried out.

The freshly passed droppings of an infected bird containing gravid segments were placed in a large glass container (10 in. diam. × 6 in. deep), the bottom of which was covered with a moist piece of turf. Twenty laboratory-bred non-infected slugs (*Agr. reticulatus*) were then put into the container. The infected droppings plus the usual diet of clean lettuce were placed in the container on three consecutive days.

After three weeks the surviving slugs were dissected. Twelve slugs remained at the end of the experiments and only five of these were found to be harbouring cysticercoids. This experiment was repeated again and of the twenty original slugs used only ten were alive after three weeks. Four of these surviving slugs were found to be infected and one dead slug although it appeared to be putrified, was found to be harbouring living cysticercoids.

Summary and Conclusions

1. The life-cycle of the fowl tapeworm *Davainea proglottina* and its various intermediate hosts are described briefly.

2. Studies on the different species of slugs in Great Britain under natural conditions were carried out and a short, simple method of differentiating the species has been presented.

3. A satisfactory method of keeping and breeding these slugs under laboratory conditions is described.

4. Feeding experiments of the different slugs on freshly collected gravid proglottides confirm that the intermediate hosts, as published by Grassi and Rovelli are as follows : *Agriolimax agrestis*, *Limax cinereus*, *Limax flavus* ; by Wetzel, *Arion empericorum* (*A. ater*), *Arion hortensis*, *Arion circumscriptus* and *Arion intermedius*. In addition to these known intermediate hosts, the writer succeeded in infecting the following species : *Agriolimax reticulatus*, *Agriolimax carvanae*, *Milax gracilis* and *Milax sowerbyi* which may accordingly serve under natural conditions as intermediate hosts for *D. proglottina*.

5. The common field or grey slug (*Agr. reticulatus*) and the garden slug (*Arion hortensis*) seem to be the most important intermediate hosts for *D. proglottina* in Great Britain. Slugs of nearly every age of these two species were experimentally exposed to infection with almost 100% positive results. Both these slugs are the most prevalent and widely distributed species in this country. This fact and the wet climatic conditions which obtain in this country are thus very favourable factors in the propagation of the tapeworm.

6. Slugs of different ages were experimentally infected and it was found that younger ones were more susceptible, but *Agriolimax reticulatus* of almost all ages became infected.

7. From the last experiment it may be concluded that slugs will feed on the droppings of fowl despite the presence of a sufficient normal diet, in this case clean lettuce.

Acknowledgment

The writer is greatly indebted to Professor J. J. C. Buckley and Dr. P. L. Le Roux for their aid, criticism and suggestions.

References

Barnes, H. F. and Weil, J. W., 1944.—"Slugs in gardens : their numbers, activities and distribution, part I." *J. Anim. Ecol.*, **13**, 140–175. (W.L. 11027a).

———1945.—"Slugs in gardens : their numbers, activities and distribution, part 2." *J. Anim. Ecol.*, **14**, 71–105. (W.L. 11027a).

Bayon, H. P., 1933.—"Recent advances in our knowledge of poultry diseases. Three lectures at the Imperial College of Science. Lecture I." *Vet. Rec.*, **13**, 655–669. (W.L. 22523).

Bisset, N., 1928.—"Observations on *Davainea proglottina* in the domestic fowl, with a note on *Amoebotaenia sphenoides* v. Linstow." *Vet. J.*, **84**, 19–32. (W.L. 22518).

Brown, F. J., 1933.—"Life history of the fowl tapeworm, *Davainea proglottina*." *Nature, Lond.*, **131**, 276–277. (W.L. 14900).

Carrick, R., 1942.—"The grey field slug *Agriolimax agrestis* L., and its environment." *Ann. appl. Biol.*, **29**, 43–55. (W.L. 1025).

Chandler, A. C., 1923.—"Observations on the life cycle of *Davainea proglottina* in the United States." *Trans. Amer. micro. Soc.*, **42**, 144–147. (W.L. 21400).

Grassi, G. B. and Rovelli, G., 1889.—"Embryologische Forschungen an Cestoden." *Zbl. Bakt.*, **5**, 370–377; 401–410. (W.L. 23684).

Joyeux, C., 1920.—"Cycle évolutif de quelque cestodes. Recherches expérimentales." *Bull. biol., Suppl.* 2, pp. 1–219. (W.L. 3919).

Kalkus, J. W., 1928.—"A preliminary note regarding the presence of *Davainea proglottina* in Washington." *J. Amer. vet. med. Ass.*, **73**, 612–616. (W.L. 11022).

Lebour, M. V., 1915.—"Some feeding habits of slugs." *Ann. appl. Biol.*, **1**, 393–395. (W.L. 1025).

Levine, P. P., 1938.—"Observations on the biology of the poultry cestode *Davainea proglottina* in the intestine of the host." *J. parasit.*, **24**, 423–431. (W.L. 11428).

Meggitt, F., 1916.—"A contribution to the knowledge of the tapeworms of fowls and of sparrows." *Parasitology*, **8**, 390–410. (W.L. 16035).

Miles, H. W., Wood, J. and Thomas, J., 1931.—"On the ecology and control of slugs." *Ann. appl. Biol.*, **18**, 370–399. (W.L. 1025).

Quick, H. E., 1947.—"*Arion ater* (L.) and *A. rufus* (L.) in Britain and their specific differences." *J. Conch.*, **23**, 249–291. (W.L. 11138).

Quick, H. E., 1949.—"Synopses of the British Fauna No. 8 slugs (Mollusca)". Linnean Society of London, pp. 1–29.

Railliet, A. and Lucet, A., 1892.—"Sur le *Davainea proglottina* Davaine." *Bull. Soc. Zool.*, **17**, 105–106. (W.L. 5401).

Riley, W. A. and James, L. G., 1922.—"Life history and methods of control of the chicken nematode (*Heterakis papillosa* Bloch)." *Rep. Minn. agric. Br. Stag.* 30th (1921–22), pp. 70–71. (W.L. 18376).

Sawyer, C. E. and Hamilton, C. H., 1929.—"Tapeworm in fowl". *Rep. W. Wash. Exp. Sta.*, (1928–29), pp. 44–45. (W.L. 18655a).

Taylor, E. L., 1933.—"*Davainea proglottina* and diseases in fowls. The pathogenicity of the common poultry parasites. An unknown factor in the causation of disease". *Vet. J.*, **89**, 500–504. (W.L. 22518).

Taylor, J. W., 1906.—"I Monograph of the land and freshwater Mollusca of the British Isles. Testacellidae, Limacidae, Arionidae. Pt. 12", pp. 225–280. Taylor Bros., Leeds.

Wetzel, R., 1932.—"Zur Kenntniss des weniggliedrigen Huhnerbandwurmes *Davainea proglottina*." *Arch. wiss prakt. Tierheilk.*, **65**, 595–625. (W.L. 1818).

A B

Important intermediate lists for *D. proglottina* in Great Britain :
A. *Agriolimax reticulatus*. B. *Arion hortensis*.

Journal of Helminthology, Vol. XXX, *No.* 4, 1956, *pp.* 203-210.

On *Kalicephalus hongkongensis* n.sp. from *Elaphe moellendorffi* and the Erection of a New Genus, *Kalicephaloides.*

By Yeh Liang-Sheng, Ph.D.
From the Department of Parasitology,
London School of Hygiene and Tropical Medicine

The present paper reports on two species of *Kalicephalus*, the first being *K. indicus* which was collected by the writer from snakes many years ago in Canton and the second *K. hongkongensis* n.sp. from a Hongkong snake which died in the London Zoo. The writer is much indebted to Dr. L. Harrison Matthews for kindly making collection facilities available at the Prosectorium of the London Zoo, and to Professor J. J. C. Buckley for his keen interest in this work.

Hsu (1934) wrote an excellent paper on *Kalicephalus* species from China, which were fairly representative of that country, as his collection covered many parts of China. He described six species, three known and three new forms : *K. chungkingensis* H. F. Hsu, 1934, *K. gongylophis* Maplestone, 1931, *K. naiae* Maplestone, 1931, *K. indicus* Ortlepp, 1923, *K. nankingensis* H. F. Hsu, 1934 and *K. sinensis* H. F. Hsu, 1934. A few years later H. W. Wu amd Y. T. Hu, (1938) in a survey of the parasitic nematodes from Hainan Island, reported three species, two known and one new form : *K. sinensis* H. F. Hsu, 1934, *K. naiae* Maplestone, 1931 and *K. assimilis* H. W. Wu and Y. T. Hu, 1938.

The genus *Kalicephalus* has a very wide distribution. The writer has found it in almost every snake examined from Canton, and in snakes at the London Zoo which came from Asia, Africa and America.

Kalicephalus indicus Ortlepp, 1923

Hsu (1934) has found this species to be very common in China, and has reported it from a large number of hosts. The present writer found it to be extremely common in the snake *Pytas mucosus*

in the markets in Canton. As the identification of species of *Kalicephalus* is not a very easy matter, I propose to give a brief description and drawing of my material.

These are medium-sized worms. The male measures 5.2–6.1 mm. in length and 0.18–0.28 mm. in maximum breadth, while the female is 6.2–7.9 mm. in length and 0.18–0.28 mm. in width. The diameter of the head at the level of the base of the buccal capsule, dorso-ventrally, is 0.17–0.21 mm. in the male and 0.18–0.24 mm. in the female. The buccal capsule in the same direction is 0.14–0.15 mm. in width and 0.13–0.15 mm. in height in the male, and 0.18 mm. in width, and 0.14–0.15 mm. in height in the female. The nerve ring and excretory pore in the male measure 0.18–0.19 mm. and 0.33–0.35 mm. respectively from the anterior end of the worm, or 0.06–0.07 mm. and 0.20–0.23 mm. from the anterior end of the oesophagus. In the female they measure 0.20–0.23 mm. and 0.41 mm. respectively from the anterior end of the worm, or 0.07–0.08 mm. and 0.29 mm. respectively from the oesophagus. The oesophagus is 0.27–0.30 mm. in length and 0.13–0.14 mm. in maximum width at the base, for the male, and 0.30–0.31 mm. and 0.14–0.18 mm. respectively for the female.

In the male the spicules are equal and measure 0.354–0.375 mm. in length, the gubernaculum 0.110–0.125 mm. and the telamon 0.046 mm.

In the female the tail is 0.13–0.15 mm. long. The vulva is 1.4–1.8 mm. from the posterior end and divides the worm in the ratio of 3.4 : 1.

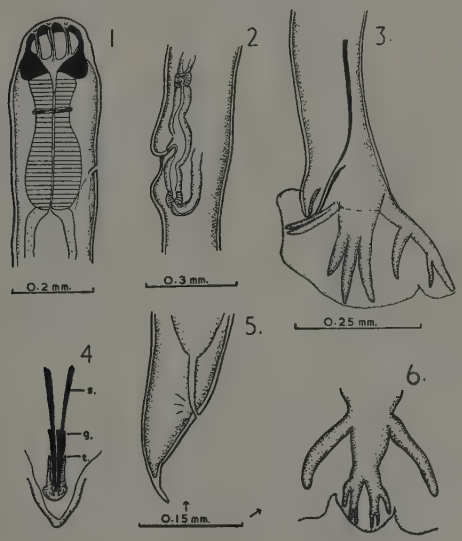
Kalicephalus hongkongensis sp.nov.

Several specimens of both sexes were collected from two snakes, *Elaphe moellendorffi*, which came from Hong Kong and died in the London Zoological Gardens. For this worm from this rare snake, I propose the name *K. hongkongensis*.

These are small worms. They are thicker anteriorly and taper away posteriorly. The transverse cuticular striations are very fine and faint.

The worms measure 2.8–3.1 mm. in length and 0.21–0.33 mm. in maximum breadth in the male and 3.7–4.1 mm. in length and

0.29–0.33 mm. in maximum breadth in the female. The head measures 0.19–0.20 mm. dorso-ventrally in the male and 0.23–0.26 mm. in the female. In the same direction, the buccal capsule measures 0.15–0.19 mm. in width and 0.14–0.15 mm. in height for the male and 0.20–0.23 mm. and 0.17–0.19 mm. for the female.



Kalicephalus indicus.

Fig. 1.—Lateral view of anterior end. Fig. 2.—Vulval region of worm. Fig. 3.—Lateral view of posterior end of male. Fig. 4.—Ventral view of genital cone with telamon and gubernaculum. Fig. 5.—Female tail. Fig. 6.—Dorsal ray pattern.

S.—spicule. G.—gubernaculum. T.—telamon.

The mouth opens directly anteriad and is not tilted dorsad. The nerve ring and excretory pore are 0.21–0.22 mm. and 0.23–0.27 mm. respectively from the anterior end of the worm, or 0.08–0.09 mm. and 0.08–0.09 mm. from the anterior end of the oesophagus, in the male. In the female they are 0.24–0.25 mm. and 0.27–0.32 mm. from the anterior end of the worm, or 0.07–0.08 mm. and 0.11–0.15 mm. from the anterior end of the oesophagus. In the male the oesophagus measures 0.30–0.32 mm. in length and the bulbous

posterior region of it 0.13–0.15 mm. in maximum breadth. In the female these measurements are 0.31–0.34 mm. and 0.16–0.17 mm. respectively.

The bursa of the male is fairly well developed. The rays are typical of the genus. The postero-lateral rays however, are directed slightly dorsally. Further details, and the dorsal ray pattern, are shown in the accompanying figures.

The spicules are equal and measure 0.264–0.292 mm. in length. Each spicule tapers to a very slender distal half which is often curved. The dorsally situated gubernaculum measures 0.120–0.129 mm. The lightly sclerotized butterfly-shaped telamon is situated ventrally and measures 0.065–0.071 mm.

The female tail is conical and slightly ventrally curved, measuring 0.17–0.19 mm. in length. The non-protuberant vulva opens 1.16–1.25 mm. from the posterior end of the worm and divides the worm in the ratio 2.19 : 1 to 2.58 : 1. The uteri are divergent, and continue a long distance each way before turning. The thin-shelled ovoid eggs measure 58–63 × 29–35 microns.

Discussion : This is a very small species of *Kalicephalus*. Only mature specimens were measured. The size range and some important measurements and morphological characters come close to those of *K. sinensis*. *K. hongkongensis* n.sp., however, differs considerably in the ramification of the dorsal ray, and in the size of the lateral rays (both of which resemble very much those of *K. gongylophis*) ; there are also some differences in the head region.

Cotypes : Deposited in the Helminthological collection, London School of Hygiene and Tropical Medicine.

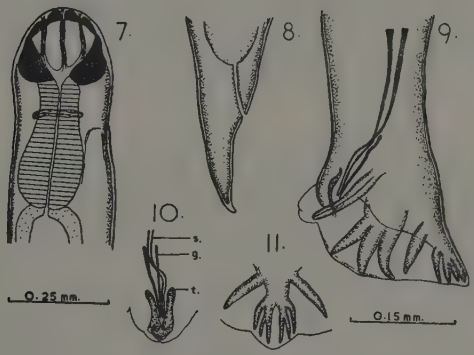
Host : *Elaphe moellendorffi*.

Locality : Hong Kong.

The Gubernaculum and Telamon in *Kalicephalus*

Since Hall first proposed the term telamon, there has been much confusion in the use of the terms gubernaculum and telamon for several nematode genera. To a certain extent this is also true for the genus *Kalicephalus*. So far, it appears that only the gubernaculum has been reported for this genus, but in at least one case, what is

described as the gubernaculum seems to me to be almost certainly the telamon (viz. Dollfus and Chabaud, 1949, page 420). The gubernaculum and telamon should not be confused. The gubernaculum which is much like a spicule in structure, is dorsal and median and formed from the spicular pouch. The telamon is immovable, ventral, light in colour, very much like the external cuticle, and formed from the cloacal lining.



Kalicephalus hongkongenisis n.sp.

Fig. 7.—Lateral view of anterior end. Fig. 8.—Female tail. Fig. 9.—Lateral view of posterior end of male. Fig. 10.—Ventral view of genital cone, showing telamon and gubernaculum. Fig. 11.—Dorsal ray pattern.

In *Kalicephalus brachycephalus* Maplestone, 1931, the same author (1932) writes : "The dorsal and ventral walls of the spicule canal are chitinized, and in addition there is a slightly curved chitinous structure in the genital cone, which is V-shaped on ventral view (fig. 49). This last structure with the chitinous ventral wall of the spicule canal are possibly the representative of a telamon, which is only partly developed in the present specimen." From this description and his drawings, it is certain that he saw the telamon, but I do not agree that it is only a "partly developed" one. In all the species of *Kalicephalus* examined, I have observed the telamon and would be rather surprised not to find a telamon in the other species of *Kalicephalus*.

A Discussion on the genus *Kalicephalus* Molin, 1861

The genus *Kalicephalus* is by no means an easy one to study, especially since many of the earlier species were improperly described. Ortlepp (1923) made a study of the genus and added to our knowledge of the group and related forms. In that work he proposed the erection of a new genus *Occipitodontus* for the reception of *Kalicephalus willeyi* v. Linstow, 1904, on the basis of "a distinct corona radiata . . . ., and also that there are three pointed teeth projecting forwards from a shallow oesophageal funnel." Baylis and Daubney (1925) made *Occipitodontus* Ortlepp, 1923, a synonym of *Kalicephalus*. Maplestone (1931) referring to Baylis and Daubney's statement writes, "their objection seems well founded, and it is followed by me in the present instance."

I have been fortunate in having the opportunity of examining the paratypes of *Occipitodontus fimbriatus* Ortlepp, 1923 and a large number of species of *Kalicephalus* collected from animals from various parts of the world dying at the London Zoo. I also had at my disposal the very extensive collection of *Kalicephalus* in the Helminthological Collection of the London School of Hygiene and Tropical Medicine. The result of studying this material makes it impossible for me to agree with Baylis and Daubney in regarding *Occipitodontus* as a synonym of *Kalicephalus*. These authors have repeatedly confused *Kalicephalus willeyi* and *Occipitodontus fimbriatus* (1922, 1923) which are not only two valid species, but entirely different in their taxonomic characters. It is probably through this confusion that they have decided to synonymise *Occipitodontus* with *Kalicephalus*. While it is true that the corona radiata is inconspicuous and not easily observed in *Occipitodontus*, the shape of the buccal capsule alone puts it into a group of its own distinct from species of *Kalicephalus*. For this reason, I feel that *Occipitodontus* is a valid genus and should be retained.

After this paper was completed and ready to go to press, it came to my notice that in the Russian *Descriptive Catalogue of Parasitic Nematodes*, Edited by K. I. Skrjabin, Vol. 3, 1952, p. 191, *Occipitodontus* is recognized as a valid genus.

Kalicephaloides gen.nov.

In the genus *Kalicephalus* the spicules are similar and equal in length, and the dorsal ray branches into two short main branches

each of which again subdivides into three terminal branches of varying pattern and length.

In *Diaphanocephalus minutus* Baylis and Daubney, 1922, which was removed to *Kalicephalus* by Ortlepp, 1923, the spicules are unequal in length and one of the terminal branches of the dorsal ray takes its origin from the base of the dorsal ray and remains widely separated for its whole length. These two characters separate this species entirely from the other species of the genus *Kalicephalus*. I propose therefore to make this species, now known as *Kalicephalus minutus* (Baylis and Daubney, 1922) Ortlepp, 1923, the genotype of a new genus *Kalicephaloides*. *Kalicephalus naiae* Maplestone, 1931, is a synonym of *Kalicephaloides minutus* (Baylis and Daubney, 1922) n.comb.

SUMMARY

Two species of *Kalicephalus* are reported from snakes. *K. indicus* is found to be very common in the snake, *Pytas mucosus* in Canton, and *K. hongkongensis* sp.nov. is reported for *Elaphe moellendorffi*.

In a large number of species of *Kalicephalus* examined, a telamon was always found to be present.

The genus *Occipitodontus* is recognised as a valid one.

Kalicephalus minutus is made the genotype of a new genus *Kalicephaloides* on the basis of the unequal spicules and one of the terminal branches of the dorsal ray having its origin at the base of the dorsal ray.

REFERENCES

BAYLIS, H. A., 1933.—"On a collection of nematodes from Malayan reptiles." *Ann. Mag. nat. Hist. Ser.* 10. **11**, 615–633. (W.L. 1050).

BAYLIS, H. A. and DAUBNEY, R., 1922.—"Report on the parasitic nematodes in the collection of the Zoological Survey of India." *Mem. Indian Mus.* **7**, 263–347. (W.L. 13401).

———— 1926.—"A synopsis of the families and genera of nematoda." 277 pp. British Museum (Nat. Hist.) London.

CABALLERO Y C. E., 1928.—"Nematodes parasites des reptiles du Mexique." *Ann. Parasit. hum. comp.*, **16**, 327–333. (W.L. 899a).

CAMPANA-ROUGET, Y. and CHABAUD, A.G., 1950.—"Note sur quelques nematodes africains collection Camille Desportes." *Ibidem*, **25**, 308–324.

COMROE, D. B., 1948.—"*Kalicephalus conoidus* n.sp. (Strongylata) a nematode from the rattlesnake *Crotalus triseriatus*". *Trans. Amer. Micr. Soc.*, **67**, 280–284. (W.L. 21400a).

DAUBNEY, R., 1923.—"Note on the genus *Diaphanocephalus* (Nematoda : Strongylidae), parasitic in reptiles, with a description of three new species." *Parasitology*, **15**, 67–74. (W.L. 10635).

DOLLFUS, R. P., and CHABAUD, A. G., 1949.—"*Kalicephalus viperae* (Rudolphi 1819) (Nematoda Strongyloidea) : un parasite de serpents à ajouter à la faune de France." *Ann. Parasit. hum. comp.*, **24**, 414–423. (W.L. 899a).

HARWOOD, P. D., 1932.—"The helminths parasitic in the amphibia and reptilia of Houston, Texas, and vicinity." *Proc. U.S. nat. Mus.*, **81**, (Art. 17), 1–71. (W.L. 16944).

———— 1934.—"Notes on Tennessee helminths. I. *Kalicephalus tennesseensis* n.sp. (Nematoda)." *J. Tenn. Acad. Sci.* **9**, 192–194. (W.L. 11575c).

HSU, H. F., 1934.—"On some *Kalicephalus* species from China with a discussion of certain systematic characters of the genus." *Peking nat. Hist. Bull.* **8**, 375–389. (W.L. 16087b).

KREIS, H. A., 1938.—"Beitrage zur Kenntnis parasitischer Nematoden VIII. Neue parasitische Nematoden aus dem Naturhistorischen Museum Basel." *Zbl. Bakt.*, **142**, 329–352. (W.L. 23684).

———— 1940.—"Beitrage zur Kenntnis parasitischer Nematoden IX. Parasitische Nematoden aus dem Naturhistorischen Museum Basel." *Ibidem.*, **145**, 163–208..

MAPLESTONE, P. A., 1931.—"Parasitic nematodes obtained from animals dying in the Calcutta zoological gardens. Parts 4–8." *Rec. Indian Mus.* **33**, 71–171. (W.L. 17746).

———— 1932.—Ibid. Parts 9–11. *Idem.* **34**, 229–261.

REIBER, R. J., BYRD, E. E. and PARKER, M. V., 1940.—"Certain new and already known nematodes from amphibia and reptilia." *Lloydia* **3**, 125–144. (W.L. 12341b).

WU, H. W. and HU, Y. T., 1938.—"Parasitic nematodes from Hainan". *Sinensia* **9**, 275–297. (W.L. 20160e).

YAMAGUTI, S., 1935.—"Studies on the helminth fauna of Japan. Part 11 Reptilian nematodes." *Jap. J. Zool.* **6**, 393–402. (W.L. 10881w).

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On Some Helminths from a King Cobra in Malaya including *Occipitodontus edesoni* n.sp. and *Ophiotaenia kuantanensis* n.sp.

By Yeh Liang-Sheng, Ph.D.

From the Department of Parasitology, London School of Hygiene and Tropical Medicine

The present collection of helminths from a King-Cobra, *Naja hannah* from Kuantan, was made by Dr. J. F. B. Edeson of the Institute of Medical Research, Kuantan, Malaya, and through the courtesy of Professor J. J. C. Buckley, the material was kindly placed at my disposal for study.

The collection consists of one species of trematode, *Styphlodora persimilis*; two species of cestodes, *Bothridium pithonis* and *Ophiotaenia kuantanensis* n.sp., and three species of nematodes, *Tanqua gigantica*, *Occipitodontus fimbriatus*, and *O. edesoni* n.sp.

Styphlodora persimilis Nicoll, 1914

Large numbers of these flukes were recovered from the ureters of a King-Cobra, *Naja hannah* from Malaya. This is a new host record for this parasite which was first reported from the ureters of an Indian River-Snake, *Tropidonotus piscator* which died at the London Zoo. Since then, I have had occasion to examine several specimens coming from this type host.

The mature specimens measure 2.3–2.9 mm. in length and 0.60–0.82 mm. in maximum breadth. Its entire cuticle is covered with prominent spines. The subterminal oral sucker measures 0.19–0.24 mm. in diameter, while the larger ventral sucker measures 0.23–0.28 mm. It is situated slightly posterior to the bifurcation of the caeca, and 0.6–0.7 mm. from the anterior end of the worm.

There is a short pre-pharynx. The pharynx is 0.12–0.13 mm. in length and the oesophagus 0.14–0.17 mm. long. The caeca are about equal in length, ending about 0.4–0.5 mm. from the posterior end. The outline of each caecum is often undulating, especially on the lateral margin.

The two testes have practically smooth borders. The anterior testis on the left measures 0.21–0.33 mm. while the larger posterior

testis on the right measures 0.25–0.40 mm. in diameter. There is a small space between the two testes in which the uterus descends and ascends. The large crescent-shaped cirrus-sac ends posterior to the hind border of the ventral sucker, and measures about 0.6–0.7 mm. in length. The genital pore opens in the median field immediately anterior to the ventral sucker.

The smooth, almost spherical ovary is situated slightly posterior to the right side of the ventral sucker, and measures 0.17–0.21 mm. in diameter. Behind it lies a large seminal receptaculum. The vitellaria are situated lateral to the intestinal caeca, extending from the middle of the ventral sucker to the middle of the anterior testis, covering a distance of about 0.4–0.7 mm. The voluminous uterus descends and ascends intercaecally, but posterior to the terminus of the caeca it fills up the entire caudal space. The eggs measure 46–50 by 21–25 microns.

Discussion. An important difference in our specimens is the undulation of the caeca, which although not described by Nicoll, is shown in his drawings. Our specimens show that this feature of the caeca is often noted, but not a constant character. Nicoll, states that the ventral sucker is "invariably transversely elongated". We have found both the oral and ventral suckers to vary from round to elongate both in our present specimens and in some of the comparative material collected from the type host, and we therefore pay no significance to their shape. However, it was always noted, as Nicoll also observed, that the ventral sucker was the larger of the two.

Bothridium pithonis Blainville, 1824

Numbers of these tapeworms were collected from the large intestine. Meggitt (1931) recorded *Bothridium* sp. encysted in *Naja naja* from Burma. The present finding is a new host record.

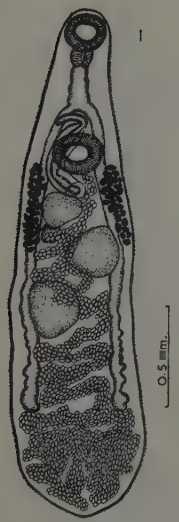
Ophiotaenia kuantanesis n.sp.

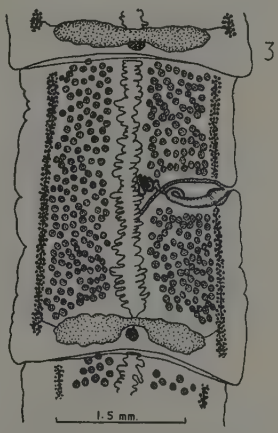
Several of these cestodes were available for study. The average specimen has about 100 segments, measures 100 mm. in length and 3 mm. in width. The mature proglottid measures 1.8 mm. long and 3.0 mm. wide, while a gravid segment measures 4.2 mm. long and 2.7 mm. wide. The neck is stout and broad, and measures 2.4 mm. The immature segments appear very gradually and are numerous.

The unarmed scolex is oblong and thus unlike the other *Ophiotaenia* species. The long axis is dorso-ventral, and measures

2.8 mm. There are four aspinose suckers pointing directly outwards, that is, laterally. The sucker has a diameter of 0.67–0.69 mm. There is no apical sucker.

The genital aperture is marginal, devoid of papillae, and alternates irregularly, with one, two, or three apertures on the same side.





Styphlodora persimilis Nicoll, 1914 Fig. 1.—Ventral view.

Ophiotaenia kuantanensis n.sp. Fig. 2.—Scolex. Fig. 3.—Gravid proglottid.

There are about 250–350 testes, average diameter 0.12 mm. and separated into two lateral fields. The vas deferens has a number of coils, but once the cirrus-sac is reached, it becomes quite straight. The cirrus-sac measures about 0.64 mm. in length, and has a ratio of its length to the breadth of the proglottid as 1 : 4.7. The cirrus is unarmed. The lateral genital pore opens slightly anterior to the middle of the segment. The vagina may open either anterior or posterior to the cirrus. The fine follicular vitellaria are arranged in two narrow columns occupying the lateral fields. The bilobed ovary lies far in the posterior field of the segment, and measures 1.8 mm. wide, and 0.35 mm. high. The uterus is central and reaches to the

anterior border of the proglottid. It has about 35–40 diverticulae. The spherical eggs measure about 33 microns in diameter.

Discussion. The present species appears allied to *Ophiotaenia grandis* La Rue, 1911, but differs considerably in the oblong shape of the scolex ; in the suckers which are twice as large ; in the smaller size of the proglottids ; in the more anterior position of the genital aperture and in the more numerous testes.

Tanqua gigantica C. C. Kung, 1948

A number of specimens of this species was collected from the stomach. This species was first described by Kung from the intestine of *Python reticulatus* from East Indies, and the oesophagus of *Naja hannah* from South East Asia.

Occipitodontus Ortlepp, 1923

In an earlier paper, the author (Yeh, 1956) showed that the genus *Occipitodontus* is a valid one. In the present material, we have found two species belonging to this genus, namely *Occipitodontus fimbriatus* Ortlepp, 1923 in the stomach, and *O. edesoni* n.sp. in the intestine.

Occipitodontus fimbriatus Ortlepp, 1923

Four males and one female specimen were collected from the stomach of *Naja hannah*. This is a new host record for this species.

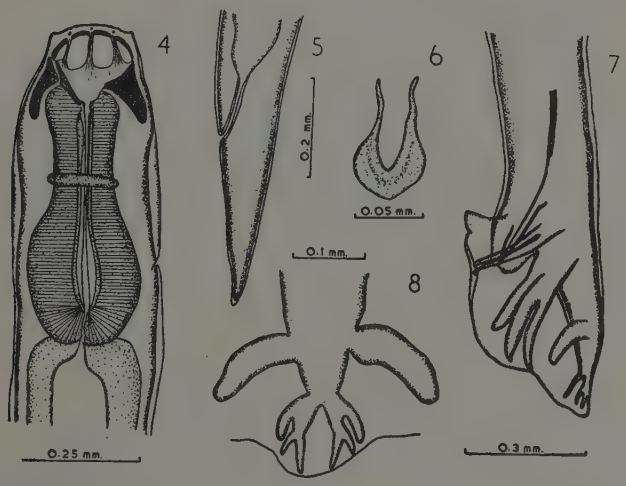
Our material differs slightly from the holotype and paratypes which were re-examined. The bursal rays are stouter than those of the types. The dorsal ray is divided into two parts, and each is again subdivided into three. The most dorsal two of these subdivisions appear to vary, and they may or may not be distinctly divided. The spicules are longer than those of the types, measuring 0.67–0.70 mm. In spite of these differences, it appears to me that they are merely variations within the species, and they are accordingly assigned to the above species.

Occipitodontus edesoni n.sp.

A large number of these worms were collected. The cuticle is finely striated. The mouth opens directly anteriad.

Male. The males measure 7.3–8.0 mm. in length by 0.24–0.31 mm. in maximum breadth. The head dorso-ventrally is 0.24–0.28 mm. The buccal cavity is 0.13–0.14 mm. wide. The buccal capsule has a

height of 0.18–0.19 mm. and 0.22–0.25 mm. dorso-ventrally at its most posterior tip. The excretory pore and nerve ring are 0.28–0.35 mm. and 0.15–0.19 mm. respectively from the anterior end of the oesophagus. The oesophagus is slightly enlarged anteriorly, but bulbous posteriorly. It has a length of 0.46–0.52 mm. and a maximum width of 0.17–0.23 mm. The oesophageal teeth are poorly developed and ill-defined. The tubular, alate spicule is 0.445–0.460 mm. in length, the gubernaculum 0.162–0.188 mm. and the



Occipitodontus edesoni n.sp.
Fig. 4.—Anterior end of worm. Fig. 5.—Tail of female. Fig. 6.—Telamon. Fig. 7.—Bursa of male. Fig. 8.—Dorsal ray.

telamon 0.08–0.09 mm. The genital cone is well developed and measures about 0.13 mm. in length. The bursa is large and the rays stout. Their pattern can be seen in the accompanying figures.

Female. The female is 8.3–9.8 mm. in length and 0.33–0.42 mm. in maximum width. The head dorso-ventrally is 0.30–0.34 mm. The buccal cavity is 0.15–0.20 mm. The buccal capsule has a height of 0.20–0.23 mm. and 0.26–0.29 mm. dorso-ventrally at its most posterior tip. The excretory pore and nerve ring are 0.30–0.38 mm. and 0.18–0.22 mm. respectively from the anterior end of the oesophagus. The bulbous oesophagus has a length of 0.52–0.55 mm. and a maximum breadth of 0.23–0.28 mm. The straight

conical tail is 0.28–0.36 mm. in length. The vulva is 2.6–3.1 mm. from the caudal end, and divides the body in the ratio of 1 : 2.1–2.6. The eggs measure about 46 by 88 microns.

This species is named in honour of Dr. J. F. B. Edeson of the Institute of Medical Research, Kuantan, Malaya.

Host : *Naja hannah*

Habitat : Small intestine

Locality : Kuantan, Malaya.

Type and paratypes : In the Helminthological collection, London School of Hygiene and Tropical Medicine.

Discussion. This species differs from the genotype by its smaller size, much thinner cuticle compared with that of the stomach-resident genotype, the more posterior position of the excretory pore and the inconspicuous oesophageal teeth.

Summary

A collection of helminths from a King-Cobra, *Naja hannah* from Malaya is described. It consists of one species of trematode from the ureter, *Styphlodora persimilis* ; two species of cestodes in the intestine, namely *Bothridium pithonis* and *Ophiotaenia kuantanensis* n.sp., and three species of nematodes, *Tanqua gigantica* and *Occipitodontus fimbriatus* from the stomach, and *O. edesoni* n.sp. from the small intestine. With the exception of *Tanqua gigantica* all the above named species are new records for the King-Cobra.

References

Kung, C. C., 1948—"On some new species of spirurids from terrestrial vertebrates, with notes on *Habronema mansioni*, *Physaloptera paradoxa* and *Hartertia zuluensis*". *J. Helminth.*, **22**, 141–164 (W.L. 11224c).

La Rue, G. R., 1914.—"A revision of the cestode family Proteocephalidae." *Illinois biol. Monogr.*, **1**, 1–350 (W.L. 9822).

Meggitt, F. J., 1931.—"On cestodes collected in Burma. Part II." *Parasitology*, **23**, 250–263 (W.L. 16035).

Nicoll, W., 1914.—"Trematode parasites from animals dying in the Zoological Society's gardens during 1911–1912." *Proc. Zool. Soc. Lond.*, pp. 139–154 (W.L. 16969b).

Ortlepp, R. J., 1923.—"Observations on the nematode genera *Kalicephalus*, *Diaphanocephalus*, and *Occipitodontus* g.n., and on the larval development of *Kalicephalus philodryadus* sp.n." *J. Helminth.*, **1**, 165–189 (W.L. 11224c).

Yeh, Liang-Sheng, 1956.—"On *Kalicephalus hongkongensis* n.sp. from *Elaphe moellendorffi* and the Erection of a New Genus *Kalicephaloides*." *Ibid.*, **30**, 203–210.

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*On Three New Species of Strigeid Trematodes from an African Crocodile and the Erection of a New Family, Neostrigeidae

By B. Bisseru, M.Sc., Ph.D.

From the Department of Parasitology
London School of Hygiene and Tropical Medicine

From the intestine of *Crocodilus niloticus*, from the Kafue River, Northern Rhodesia, some interesting strigeid trematodes were recovered which have been placed in a new family. These forms which at a cursory examination could be taken to resemble the avian parasites of the genus *Strigea*, are of particular interest since they have not been known before as parasites of the Reptilia. The present material, which was made available through the kindness of Dr. P. L. LeRoux, comprises three new species for which two new genera are formed.

Neostrigeidae fam.nov.

Neostrigea gen.nov.

Neostrigea africana sp.nov.

Several specimens of this trematode were taken from the intestine of the African Crocodile, *Crocodilus niloticus*, which also harboured two specimens of *Neostrigea leiperi* gen.et sp.nov. and about two dozen specimens of *Prostrigea arcuata* gen.et sp.nov.

The trematodes are more or less cylindrical in shape. The body is considerably arched in the fixed state, with the posterior end of the body in most cases tending to touch the anterior body in the region of the ventral sucker. The posterior segment is mainly concerned with this arching of the body.

The mature worm measures 2 to 2.7 mm. in length. The body has a smaller anterior segment, ovoid to somewhat spherical in shape, measuring 0.7–0.8 mm. long × 0.54–0.65 mm. wide. Of the lobes of the holdfast organ the largest one projects to the exterior

*Part of a Thesis approved by the University of London for the award of the Ph.D. degree.

ventrally. The anterior body is separated by a transverse constriction from the hindbody, which is 1.45–1.90 mm. long × 0.56–0.68 mm. wide in the region of the ovary, decreases in width posteriorly being about 0.33–.042 mm. across the bursa copulatrix. The posterior extremity is broadly rounded. The ratio of the length of the posterior segment to the anterior one is about 2:1.

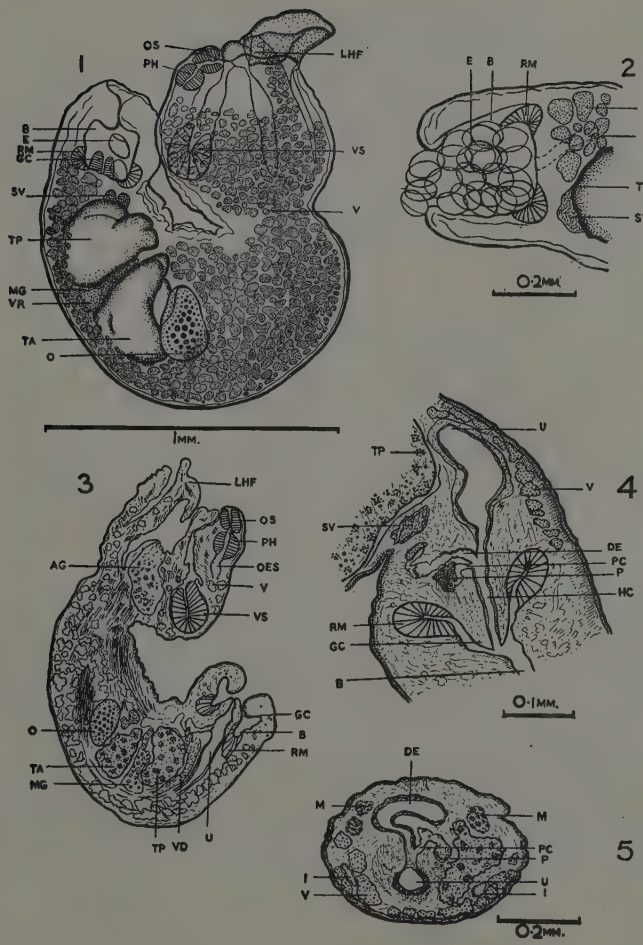
The oral sucker measures 0.1–0.115 mm. × 0.09–0.13 mm. Viewed in saggital section, one half of the sucker is characteristically smaller than the other half (Fig. 3). A very short prepharynx is discernible, and is seen more clearly in serial sections. The dimensions of the muscular pharynx are 0.08–0.096 mm. long × 0.093–0.112 mm. wide. A short oesophagus bifurcates into the intestinal caeca, which pass into the hindbody ending a short distance from its posterior extremity. The ventral sucker is 0.148–0.22 mm. long × 0.1–0.17 mm. wide. It lies dorsally at the bottom of the internal depression of the anterior segment. The adhesive gland is either ventral or postero-ventral to the ventral sucker and in *in toto* mounts of mature forms its outline is difficult to discern owing to the opaqueness caused by the vitelline follicles in this part of the body. In serial sections the adhesive gland shows closely packed groups of gland cells. It measures 0.2–0.24 mm. antero-posteriorly by 0.166–0.19 mm. transversely.

The cuticle is unarmed. The parenchyma of the body is traversed by numerous bands of longitudinal muscle fibres which originate in the forebody, mainly at the base of the internal depression, and continue into the hindbody as far as the bursa copulatrix, in which part the transverse diameter of the muscle fibres is 0.04–0.09 mm.

The lobed testes lie one behind the other and occupy mainly the posterior part of the hindbody. The size of the anterior and posterior testes are 0.24–0.36 mm. long × 0.225–0.4 mm. wide and 0.3–0.4 mm. long × 0.32–0.39 mm. wide respectively. Both testes are concave,

Abreviations Used

AG=adhesive gland; **B**=bursa copulatrix; **DE**=ejaculatory duct; **E**=egg; **GC**=genital cone; **HC**=hermaphrodite canal; **I**=intestine; **LHF**=lobes of holdfast organ; **LP**=lumen of paraprostate gland; **M**=longitudinal muscle fibres; **MG**=Mehlis' gland complex; **O**=ovary; **OES**=oesophagus; **OLC**=opening of Laurer's canal; **OS**=oral sucker; **P**=paraprostate; **PC**=prostatic canal; **Ph**=pharynx; **RGC**=retracted genital cone; **RM**=ring muscle of bursa; **SR**=seminal reservoir; **SV**=seminal vesicle; **TA**=anterior testis; **TP**=posterior testis; **U**=uterus; **V**=vitellaria; **VD**=vas deferens; **VR**=vitelline reservoir; **VS**=ventral sucker.



Neostrigea africana gen.et sp.nov.

Fig. 1.—Adult parasite. Fig. 2.—Posterior end of body, showing bursa with eggs. Fig. 3.—Longitudinal section through body. Fig. 4.—Longitudinal section showing paraprostate and genital ducts in region of bursa copulatrix. Fig.5.—Cross section showing association of paraprostate with genital ducts.

the concavity being directed dorsally. A large seminal reservoir between the ovary and anterior testis characterizes almost all specimens. The swollen and coiled vas deferens travels posteriorly from the seminal reservoir, passing dorsal to the uterus and ventral to both testes, giving rise to a well developed vesicula seminalis posterior to the hind testis. The ejaculatory duct enters the uterus from the dorsal side outside the genital cone and bursa copulatrix. The muscular hermaphrodite duct opens to the exterior within a spacious bursa copulatrix, 0.136–0.18 mm. long × 0.175–0.3 mm. wide, after traversing a genital cone which projects into the bursa. The maximum depth of the bursa was found to be 0.32 mm. The bursal ring muscles have a diameter of 0.03–0.05 mm. A dorsally-placed, weakly muscular para-prostate is present containing numerous gland cells. The prostatic canal joins the ejaculatory duct, and the common male duct so formed is 0.035–0.045 mm. long, and leads into the uterus. The hermaphrodite duct (ejaculatory duct, prostatic duct and uterus) has a length of 0.1–0.14 mm. A cirrus is absent.

The ovary like the testes is concave with the concavity facing dorso-posteriorly. It lies at about the 31st to 43rd hundredths of the length of the posterior segment and has an antero-posterior diameter of 0.15–0.19 mm. The Mehlis' gland complex and vitelline reservoir are inter-testicular. A Laurer's canal is present. A receptaculum seminis was not observed. The uterus passes anterior to the ovary, and in its course posteriorly, it passes ventral to the genital organs. The uterine walls dilate and at the same time become very muscular. The eggs, 0.086–0.105 mm. long × 0.06–0.07 mm. wide, seem to be contained temporarily within the bursa, and in one specimen 22 eggs were counted in this site. The follicular vitellaria extend into both body segments. In the anterior segment the follicles reach midway between the level of the pharynx and acetabulum on the dorsal side, and ventrally the follicles enter the projecting lobes of the holdfast organ for a short distance. The follicles are massed in the area immediately anterior to the ovary in the posterior segment, and in the post-ovarian area the glands are ventrally directed towards the bursa copulatrix.

Host : *Crocodilus niloticus.*

Habitat : Intestine

Locality : Kafue River, Northern Rhodesia.

Types : Department of Parasitology, London School of Hygiene and Tropical Medicine.

Neostrigea leiperi sp.nov.

Only two specimens, both mature, of this trematode were recovered from the intestine of *Crocodilus niloticus*.

The preserved worms, which are cylindrical and arched, are 3.5 mm. and 4 mm. long respectively. Both the anterior and posterior extremities of the body are blunt. The thickness of the body in the region of the posterior testis is about 0.8–0.9 mm. The heavy concentration of vitelline follicles especially in the anterior segment and in the pre-ovarian part of the posterior segment makes the body opaque.

The anterior or forebody is 0.94–1.02 mm. long × 1–1.18 mm. broad. The maximum projection of the lobes of the holdfast organ is about 0.32 mm. from the anterior border of the forebody. The posterior or hindbody which is 2.56–2.96 mm. long and about 1.2 mm. across the testicular region, is separated from the anterior body by a transverse constriction measuring 0.5–0.65 mm. The width of the body in the region of the bursa is 0.48–0.56 mm. The ratios of the length of the posterior segment to the anterior one in the two specimens are 1:2.73 and 1:2.94 respectively.

The oral sucker at the apex of the dorsal surface of the forebody measures 0.122–0.154 mm. long × 0.14–0.16 mm. wide. It is followed by a very short prepharynx, which leads to a muscular globular pharynx 0.1–0.12 mm. long × 0.133–0.151 mm. wide. The ventral sucker lying dorsally at the base of the depression of the anterior segment is not observed in *in toto* preparations on account of the thickness of the body and the opaqueness caused by the concentration of vitellaria in this part. In longitudinal sections its dimensions are 0.216 mm. × 0.155 mm. The intestinal caeca terminate a short distance from the end of the body. The adhesive gland lies posterior to the ventral sucker and its posterior border has been found to overlap slightly the antero-posterior body constriction. Its measurements are 0.245 mm. long × 0.175 mm. wide.

The cuticle is smooth. The longitudinal muscle fibres arising in the forebody are well developed and continue into the hindbody as far as the bursa copulatrix.

The two testes lie one behind the other, posterior to the ovary. They are conspicuous lobed organs, having a dorsal concavity. The

anterior testis measures 0.6 mm. × 0.75–0.9 mm. wide and its posterior border is at the middle of the hindbody. The posterior testis which lies entirely in the posterior half of the hindbody, is slightly larger than the anterior testis, and measures 0.75 mm. longitudinally by 0.86–0.98 mm. transversely. A large seminal reservoir is present between the ovary and anterior testis. The seminal vesicle is a coiled structure. The muscular, sinuous, ejaculatory duct descends from the seminal vesicle and on receiving the prostatic duct from the dorsally placed muscular prostatic gland, it enters the uterus at the summit of the muscular genital cone. The prostatic gland is packed with numerous deep-staining prostate gland cells. The genital cone is retracted into the body in both specimens, and has a diameter of about 0.3 mm. The copulatory bursa is guarded by bursal ring muscles, 0.32–0.48 mm. wide. Its depth varies from 0.25–0.32 mm.

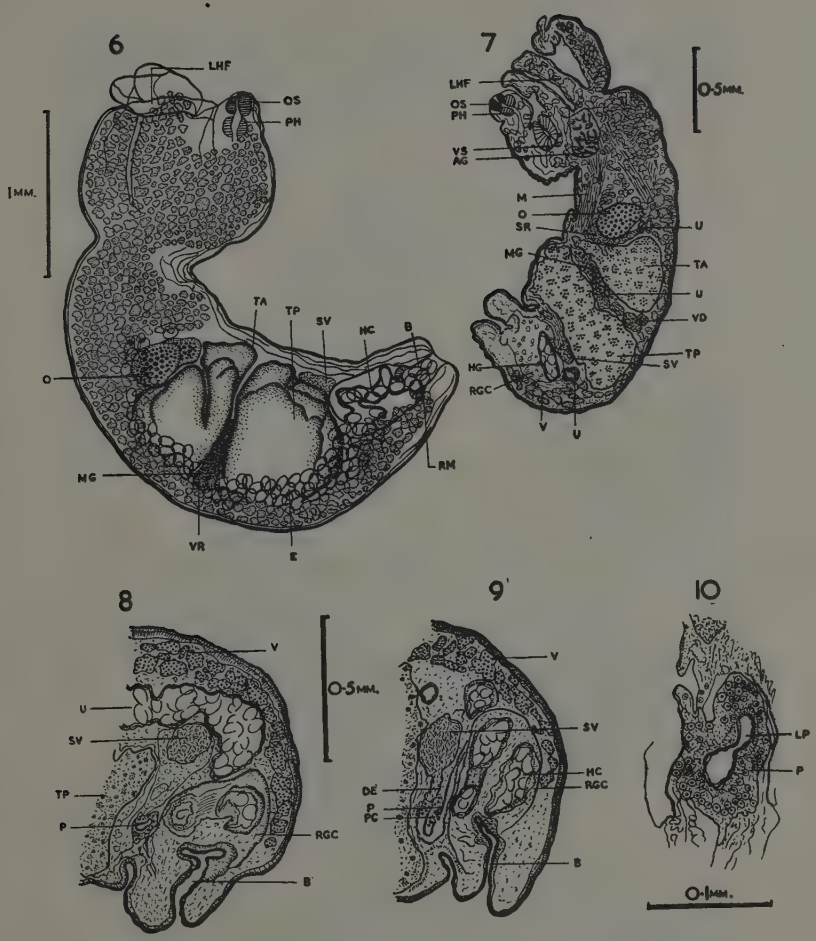
The ovary lies in the anterior third of the hindbody and like the testes has a dorsal concavity. It measures 0.23–0.26 mm. anteroposteriorly × 0.4–0.5 mm. transversely, and lies within the 25th to 33rd hundredths of the length of the hind segment. The Laurer's canal opens on the dorsal surface of the body, on a level with the posterior border of the ovary. The Mehlis' gland complex is intertesticular. The uterus on arising from the ootype is first seen in the inter-testicular space. It then passes anterior to the first testis and is immediately thrown into many coils, which are also seen anterior to the ovary. The posterior course of the uterus ventral to the reproductive organs is almost straight, but follows a somewhat sinuous course in the testicular area. Although the uterus throughout is a fairly muscular tube, its walls become thicker in the region of the seminal vesicle. Within the retracted genital cone it is coiled and opens to the exterior within the bursa copulatrix. The eggs measure 0.097–0.106 mm. long × 0.065–0.069 mm. wide. The vitellaria consist of closely-packed follicles, entering the lobes of the holdfast organ and extending to the bursa copulatrix. The follicles are situated ventrally in the post-ovarian part of the body.

Host : *Crocodilus niloticus.*

Habitat : Intestine.

Locality : Kafue River, N. Rhodesia.

Type : Department of Parasitology, London School of Hygiene and Tropical Medicine.



Neostrigea leiperi gen.et sp.nov.

Fig. 6.—Adult parasite. Fig. 7.—Longitudinal section through body. Fig. 8.—Longitudinal section through posterior part of body, showing uterus, seminal vesicle, paraprostate, retracted genital cone and bursa copulatrix. Fig. 9.—Longitudinal section through paraprostate and other genital ducts. Fig. 10.—Longitudinal section through paraprostate.

Comparison between *N. africana* and *N. leiperi*

Neostrigea africana is very similar to *Neostrigea leiperi* in the general morphology of the body, but differs from it in several important details. *N. africana* is a much smaller form in all respects, with the ratio of the length of the posterior segment to the anterior one about 1:2, in contrast to 1:3 in *N. leiperi*. Similarly the ovary is placed at about the 31st to 43rd hundredths of the length of the posterior segment in *N. africana* and the 25th to 33rd hundredths in *N. leiperi*. A notable difference between the two species is found in the terminal portions of the genital ducts and the paraprostate. *N. africana* has a small weakly muscular paraprostrate, and the prostatic canal enters the ejaculatory duct, very near the opening of this duct into the uterus. In *N. leiperi* the paraprostate is larger and more muscular, and the short prostatic duct discharges into the ductus ejaculatorius far from the opening of the latter into the uterus.

The two species resemble one another in the nature of the lobes of the holdfast organ and the relative positions of the oral sucker, pharynx, ventral sucker and adhesive organ in the first segment. The eggs in *N. leiperi* are slightly larger, and while this difference is not very great it may be relied upon for a clear-cut specific differentiation. The distribution of the vitelline follicles is very much on the same pattern in both species, but the antero-dorsal limit in *N. africana* is about half-way between the ventral sucker and pharynx, whereas in *N. leiperi* the follicles may reach up to the anterior limit of the pharynx.

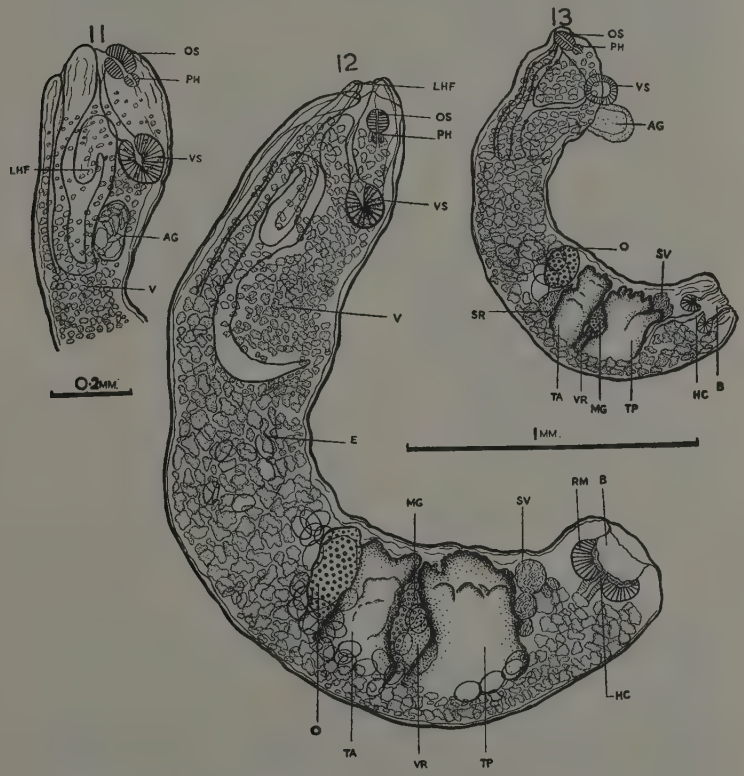
Prostrigea gen.nov.

Prostrigea arcuata sp.nov.

About two dozen specimens of this form were obtained from the intestine of the African Crocodile, *Crocodilus niloticus*.

Both mature and immature forms have a characteristic crescentic body in the fixed state. This curvature of the body is seen in every individual and the convexity is always ventral. In the preserved state these cylindrical flukes lie on their side, and any attempt to mount them dorso-ventrally on a slide has always resulted in a break at about the junction of the anterior and posterior body segments.

The anterior extremity of the body is usually blunt, but a very slight attenuation that has been observed in some specimens has been due to the disposition of the lobes of the holdfast organ as they project out of the body. The posterior extremity is blunt and



Prostrigea arcuata gen.et sp.nov.

Fig. 11.—Anterior end of worm, showing oral sucker, pharynx, ventral sucker, adhesive gland and lobes of holdfast organ. Fig. 12.—Adult parasite. Fig. 13.—Ventral sucker and adhesive gland in extruded condition.

carries the bursa copulatrix. The whole body is covered with a fairly thin cuticle which is smooth and devoid of either spines or scales. The depression of the first segment extends beyond the adhesive gland. Originating in the body wall at the base of the

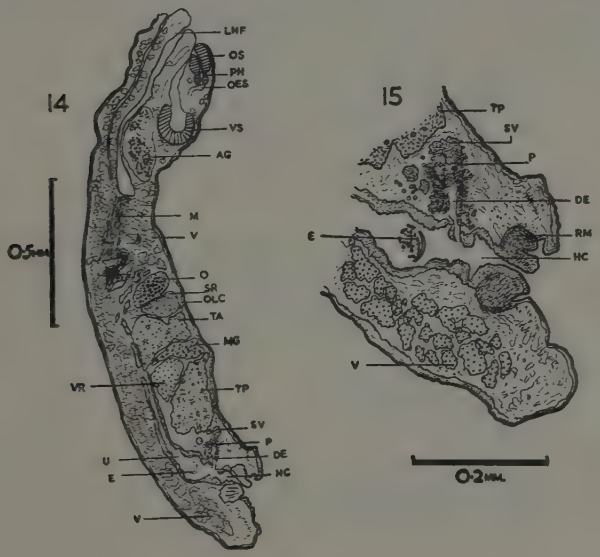
depression of the first segment are longitudinal muscle fibres which traverse most of the body length, and end in the region of the bursa.

Some adult individuals are contracted. The elongate body which at first sight does not seem to be divided into two body regions, is 1.6–3.3 mm. long (average length 2.55 mm.). A narrowing of the body takes place at the end of the depression of the first segment and this represents the end of the anterior segment, but a marked body constriction separating the two body regions is absent. A decrease in the width of the body is also seen in the region of the bursa copulatrix. The length of the first segment varies from 0.53–1.27 mm. (average 0.93 mm.) The respective widths of the body across the acetabulum and adhesive gland are 0.34–0.54 mm. and 0.25–0.58 mm. The dimensions of the posterior segment are : length 1.1–2 mm. (average 1.6 mm.) ; width 0.36–0.67 mm. in the testicular region and 0.3–0.45 mm. across the bursa copulatrix. The ratio of the length of the anterior segment to the posterior one is 1:1.6 to 2.2 (average 1:1.8).

The oral sucker is spherical to ovoid measuring 0.09–0.11 mm. long × 0.07–0.1 mm. wide. There is no prepharynx. The globular pharynx is 0.03–0.05 mm. long × 0.04–0.05 mm. wide ; the ventral sucker, measuring 0.12–0.15 mm. long × 0.12–0.14 mm. wide is dorsally placed in the depression of the first segment. The oesophagus is extremely thin, about 0.01 mm. in diameter. The intestinal caeca have not been seen either in *in toto* mounts or in serial sections. The dorsal and ventral lobes of the holdfast organ which correspond more or less to the length of the first segment arise deep down in the depression of the anterior body. The adhesive gland which is largely obscured by the vitellaria in mature individuals, lies a short distance posterior to the ventral sucker on the dorsal side of the body, but in some forms it may extend up to the side of the ventral sucker. In *in toto* preparations of young individuals it is an ovoid lobulated structure with a greater antero-posterior diameter. It measures 0.15–0.25 mm. long × 0.085–0.11 mm. wide.

The testes are ovoid to rectangular lobed bodies situated one behind the other. They occupy most of the middle third and even extend into the hind third of the posterior body. The equatorial diameter of the hindbody in almost every case passes through the anterior testis. Neither of the testes show any concavity. In lateral view the first testis is 0.22–0.3 mm. long × 0.3–0.48 mm. wide. The

second testis is slightly larger and measures 0.33–0.45 mm. × 0.38–0.54 mm. The two vasa efferentia unite in front of the anterior testis to form the vas deferens which dilates to form a large seminal reservoir between ovary and first testis. The vas deferens proceeds posteriorly ventral to testes but dorsal to uterus and enlarges into a convoluted vesicula seminalis immediately behind the hind testis.



Prostrigea arcuata gen.et sp.nov.

Fig. 14.—Longitudinal section through body. Fig. 15.—Longitudinal section through posterior end of body, showing prostate gland and genital ducts.

The seminal vesicle narrows to form the ejaculatory duct which descends ventrally to open into the muscular uterus. The hermaphrodite duct so formed has a length of 0.1–0.13 mm., and it opens on to a muscular genital cone, which projects for a short distance into the bursa copulatrix. The depth of the bursa varies from 0.12–0.2 mm.; it possesses muscular ring muscles.

The ejaculatory duct is surrounded by numerous unicellular glands, the pars prostatica—(Periprostate-Dubois). A muscular paraprostate is absent. The paraprostatic cells are massed around the ductus ejaculatorius. A cirrus is absent.

The ovary, lying anterior to the testes, measuring 0.15–0.21 mm. antero-posteriorly and 0.23–0.34 mm. transversely, has a variable position in relation to the length of the posterior segment. Its position has been calculated to lie between the 32nd to the 39th hundredths of the length of the posterior segment in normally extended specimens. In forms in which the posterior half of the hindbody has undergone some degree of contraction, the position changes to about the 38th to 45th hundredths of the length of the posterior body. The Mehlis' gland complex is inter-testicular. The Laurer's canal opens on the dorsal surface of the body. The uterus extends almost to the antero-posterior body division and in its posterior course it is sinuous in the pre-ovarian region, but posterior to this area it is a more or less straight tube, with very muscular walls. The uterine eggs measure 0.093–0.105 mm. long × 0.061–0.068 mm. wide.

The vitellaria consist of numerous irregular follicles, which extend from about the level of the oral sucker anteriorly to the bursa copulatrix posteriorly. The follicles are ventrally disposed behind the ovary and enter the wall of the bursa. The ventrally-placed inter-testicular vitelline reservoir has a longitudinal diameter of 0.12–0.14 mm.

Host : *Crocodilus niloticus*.

Habitat : Intestine.

Locality : Kafue River, N. Rhodesia.

Types : Department of Parasitology, London School of Hygiene and Tropical Medicine.

Discussion

The morphological features of the three strigeid flukes parasitic in the Crocodilia do not agree, except in the presence of a paraprostate, with the characters of the family Proterodiplostomatidae erected by Dubois (1936) for the Diplostomes of Reptiles. This family has the anterior segment of the body flat and leaf-like, the testes are spherical to ovoid without lobes, and the paraprostate is situated dorsal to the ejaculatory duct.

In studying the relationships of these Crocodilian Strigeids, it has been noted that much emphasis has been placed by Dubois on the presence or absence of a paraprostate for distinguishing the families within his subsuperfamily Diplostomatines. The material described above undoubtedly belongs to Dubois' subsuperfamily

Strigeines, in which hitherto a paraprostate was not known. In order to fit into Dubois' classification of his superfamily Strigeides it becomes necessary to erect a new family within the subsuperfamily Strigeines for the species described, though two of the species while having a paraprostate, clearly show in many respects a very close resemblance to the genus *Strigea*.

For this reason it is here proposed that these trematodes from Crocodiles should be placed in a separate family *Neostrigeidae* fam.nov., until more is known of the structure of the copulatory apparatus in many of the strigeid species, particularly those from African water-birds. (See table on *Classification of Strigeids*).

NEOSTRIGEIDAE fam.nov.

The three species are included in two new genera :—

(i) Genus *Neostrigea* gen.nov.
  (a) *Neostrigea africana* sp.nov.
  (b) *Neostrigea leiperi* sp.nov.

(ii) Genus *Prostrigea* gen.nov.
  Prostrigea arcuata sp.nov.

Family Diagnosis. Strigeids parasitic in Crocodiles. Body more or less cylindrical, curved or very much arched. Lobes of holdfast organ arise at the base of the depression of the anterior segment, and project dorsally and ventrally beyond rim of the anterior body. Testes are distinctly lobed. Vitellaria distributed in both segments. Paraprostate present. Hermaphrodite duct present.

NEOSTRIGEA gen.nov.

Generic Diagnosis. Body bisegmented with distinct transverse constriction and more or less cylindrical in outline. Anterior segment ovoid to rectangular (rarely spherical) and smaller than posterior segment. Depression of first segment does not extend beyond the middle of the adhesive gland, and contains the dorsally-placed ventral sucker. Lobes of holdfast organ project dorsally and ventrally to the exterior from the anterior border of the first segment, the largest lobe being directed ventrally. Adhesive gland is largely ventral to ventral sucker. Pharynx of about the same size as oral sucker. Ventral sucker placed posterior to middle of first segment.

Gonads in tandem position in posterior segment. Testes are lobed and concave dorsally. Vesicula seminalis convoluted behind the hind testis. Cirrus absent, ductus ejaculatorius fairly long. Paraprostate present, small, muscular and surrounded by numerous

gland cells. Paraprostatic canal discharging into ejaculatory duct, male duct so formed entering uterus outside the muscular genital cone. Hermaphrodite duct opens on genital cone into genital atrium, within bursa copulatrix. Ring muscles of bursa well developed.

Ovary ovoid, arched (dorsally concave), in front of first testis. Laurer's canal present. Mehlis' gland and vitelline reservoir inter-testicular. Vitellaria follicular in both body segments, follicles heavily concentrated anterior to ovary, reaching beyond level of pharynx and entering lobes of holdfast organ anteriorly. Vitellaria posterior to ovary ventrally situated, entering wall of bursa copulatrix. Uterine coils ascend to anterior border of ovary and descend ventrally to genital pore. Ova fairly numerous.

Type species : *Neostrigea africana* sp.nov.

Additional species : *Neostrigea leiperi* sp.nov.

Prostrigea gen.nov.

Generic Diagnosis. Small, cylindrical, crescentic or sickle-shaped strigeid flukes. Body with more or less constant width throughout. Distinct transverse constriction separating anterior segment from posterior segment lacking, but slight narrowing of body immediately posterior to depression of forebody marks end of first segment. Internal depression of forebody extends beyond adhesive gland. Anterior segment elongate, smaller than posterior segment. Elongate lobes of holdfast organ project dorsally and ventrally from anterior rim of body. Adhesive gland posterior to ventral sucker. Pharynx about half the size of oral sucker. Ventral sucker anterior to middle of first segment.

Gonads one behind the other in posterior segment. Testes lobed. Vesicula seminalis convoluted behind hind testis. Cirrus absent. Paraprostate (periprostate-Dubois) present and consists of numerous gland cells surrounding the ejaculatory duct, which opens into uterus outside genital cone. Hermaphrodite duct opens into genital atrium within bursa copulatrix. Bursal ring muscles present.

Ovary ovoid, pre-testicular. Laurer's canal present. Mehlis' gland and vitelline reservoir inter-testicular. Follicular vitellaria distributed in both body segments, not extending beyond pharynx anteriorly, and entering walls of bursa copulatrix posteriorly. Uterus almost reaches anterior limit of posterior body and opens at genital pore. Ova fairly numerous.

Type species : *Prostrigea arcuata* sp.nov.

CLASSIFICATION OF STRIGEIDS

(MODIFICATION OF TABLE GIVEN BY DUBOIS, 1953 : 24.)

STRIGEIDES

Anterior segment more or less cup-shaped. Holdfast organ in the form of two elongate retractile lips, one ventral the other dorsal.	Parasites of warm-blooded vertebrates. Paraprostate absent.	Parasites of mammals. Anterior segment much longer than posterior segment. Fam. Strigeidae.
		Parasites of birds. Anterior segment much shorter than posterior segment. Fam. Strigeidae.
	Parasites of cold-blooded vertebrates. Paraprostate present.	Parasites of reptiles. Anterior segment much shorter than posterior segment. Fam. Neostrigeidae nov. fam.
Anterior segment foliaceous, spathulate, etc. Holdfast organ variable (rounded, elliptical etc.) in outline, with or without cavity.	Parasites of warm-blooded vertebrates. Paraprostate absent.	Parasites of mammals. Development of holdfast organ large to massive (without opening). Fam. Diplostomatidae. Subfam. Alarlinae.
		Parasites of birds. Holdfast organ small to medium-size, opening generally by a median slit. Fam. Diplostomatidae. Subfam. Diplostomatinae.
	Parasites of cold-blooded vertebrates. Paraprostate present.	Parasites of crocodiles and chelonians. Holdfast organ small or medium-size, with papillae. Fam. Proterodiplostomatidae. Supersubfam. Proterodiplostomatidi.
		Parasites of snakes. Holdfast organ large, without papillae. Fam. Proterodiplostomatidae. Supersubfam. Ophiodiplostomatidi.
Anterior segment bulbous, massive, developed at the base into an equatorial cup-like pad. Holdfast organ forms a lobe transversely elongate.	Parasites of birds. Anterior segment sunken in the manner of a rostrum under mucosa of host. No oral sucker. Pharynx reduced. Digestive canal atrophied. Fam. Bolbocephalodidae.	

Comparison of the Genera *Neostrigea* and *Prostrigea*

The genera *Neostrigea* and *Prostrigea* are considered to be closely related, agreeing in the arrangement of the gonads and in the general morphology of the body. The genus *Neostrigea* is regarded as distinct from *Prostrigea* because of the more distinct bisegmentation of the body, the nature of the anterior segment and lobes of the holdfast organ, the extent of the internal depression of the anterior segment, the position of the adhesive gland in relation to the ventral sucker, the size of the pharynx in relation to the oral sucker, position of the ventral sucker in relation to the length of the anterior segment, presence of a more pronounced and muscular genital cone, more anterior extent of the vitellaria, the anterior extent of the uterus, the arched and dorsally concave nature of the ovary and testes, and in the nature of the paraprostate.

As stated before the prostatic gland (periprostate) in *Prostrigea* is in the form of a cellular mass surrounding the male ejaculatory canal into which the secretion is directly discharged. It thus differs from the prostatic gland (paraprostate) of the genus *Neostrigea*, in which this organ is independent of the male genital duct, in relation to which it is dorsal and consists of a sac-like more or less muscular reservoir surrounded by cells which discharge their secretion into it and open into the male genital duct by an efferent prostatic canal.

Summary

Neostrigea africana gen.et sp.nov., *Neostrigea leiperi* gen.et sp.nov. and *Prostrigea arcuata* gen.et sp.nov. are described from the intestine of *Crocodilus niloticus*, Kafue River, Northern Rhodesia. The three species are included in two new genera which are placed in a new family *Neostrigeidae*.

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I should like to express my thanks to Professor J. J. C. Buckley for facilities and constant advice, and to Dr. P. L. Le Roux both for his helpful criticisms and placing at my disposal his very valuable trematode collection from Central Africa. I am deeply indebted to Mr. S. Prudhoe, British Museum (Natural History) for his great kindness in allowing me to examine specimens and literature.

References

Dubois, G., 1938.—"Monographie des Strigeida (Trematoda)". *Mem. Soc. neuchatel Sci. nat.*, **6**, 535 pp. (W.L. 13318a).

————1953.—"Systematique des Strigeida, complement de la Monographie". *ibidem*, **8**, 141 pp.

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